(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 December 2024 (12.12.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/251997 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *A61P 25/28* (2006.01)

(21) International Application Number:
PCT/EP2024/065817

(22) International Filing Date:
07 June 2024 (07.06.2024)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
23178124.6 07 June 2023 (07.06.2023) EP
23212300.0 27 November 2023 (27.11.2023) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: VACCINE FOR THE TREATMENT OF AMYLOIDOSIS

(57) Abstract: Provided is a peptide-based vaccine for treating or preventing amyloid transthyretin amyloidosis (ATTR).

WO 2024/251997 A1

Vaccine for the treatment of amyloidosis

FIELD OF THE INVENTION

The present invention relates to a vaccine comprising a peptide derived from transthyretin (TTR), *i.e.,* a TTR peptide, as well as to said vaccine for use in a method for treating or preventing amyloid transthyretin amyloidosis (ATTR). More particularly, the TTR peptide comprises a neoepitope which is selectively presented and accessible, respectively, in misfolded, oligomeric and/or aggregated forms of TTR. The present invention further relates to a kit comprising said vaccine.

BACKGROUND OF THE INVENTION

Amyloid transthyretin amyloidosis (ATTR) is a severe age-associated disease leading to cardiomyopathy and/or sensorimotor polyneuropathy (Gertz *et al.*, J. Am. Coll. Cardiol. 66 (2015), 2451–24661) and includes two sub-types - wild-type ATTR (ATTRwt) and variant ATTR (ATTRv) - that vary regarding their pathogenesis. Their common precursor protein transthyretin (TTR) physiologically functions as a transport protein for thyroxin and retinol-binding protein. TTR is predominantly synthesized in the liver and occurs as a tetramer in its natural form (Alshehri *et al.*, J. Neuroendocrinol. 27 (2015), 303-3239). ATTRv, formerly known as hereditary/mutant ATTR, is an autosomal-dominant disorder. For both wild-type TTR (TTRwt) and mutant/variant TTR (TTRv) proteins, the pathogenic mechanism of ATTR is triggered by a partial unfolding of the TTR protein and subsequent aggregation into beta-pleated sheets forming amyloid fibrils (Eisele *et al.*, Nat. Rev. Drug Discov. 14 (2015), 759-780).

ATTR is characterized by two main forms of clinical presentation. Predominant amyloid fibril accumulation in cardiac tissues leads to cardiomyopathy, whereas fibril deposition in nerve fibers leads to polyneuropathy (Ando *et al.*, Guideline of transthyretin-related hereditary amyloidosis for clinicians. Orphanet J. Rare Dis. 8 (2013), 31). The factors triggering amyloid deposition in a specific organ have not been elucidated yet. Patients commonly present with a mix of symptoms, with only few TTR mutations known to exclusively cause pure cardiac or neuropathic disease (Maurer *et al.*, J. Am. Coll. Cardiol. 68 (2016), 161–172).

In the treatment of ATTR there are three current established concepts:

(i) The TTRv is almost completely replaced in the peripheral blood after orthotopic liver transplantation by the synthesis of the TTRwt in the donor liver. Beside the well-known shortages on organ availability, liver-transplanted ATTRv patients exhibit a slower but continued worsening of their condition.

(ii) Low molecular weight compounds stabilize the TTR tetramer and thereby minimize the formation of amyloid precursors. Diflunisal, AG10 and tafamidis stabilize the physiological TTR tetramer. Tafamidis has been approved for the treatment of stage 1 ATTR since 2011.

(iii) Gene silencers (mRNA-inhibiting oligonucleotides) reduce liver-secreted TTRv and TTRwt. Inotersen represents an antisense oligonucleotide that is administered subcutaneously (s.c.) once a week. Patisiran acts as a siRNA oligonucleotide administered intravenously (i.v.) every three weeks in combination with premedication. Both gene silencers were approved in 2018 for the treatment of ATTR stages 1 and 2.

Meanwhile, gene editing CRISPR/CAS9 enabling targeted *in vivo* genome editing is explored as an emerging therapy in ATTR amyloidosis in a phase I, open-label, multicenter trial with an *in vivo* gene-editing therapeutic agent, called NTLA-2001. In addition, monoclonal antibodies (mAbs) are investigated as an additional concept of potential treatment for ATTR, *e.g.*, PRX004 (NNC6019-0001), an investigational mAb designed to prevent fibril formation by specifically targeting and clearing the misfolded forms of the TTR protein found in ATTR-CM and NI006, investigational human mAb that targets TTR amyloid and most recently has been shown in a Phase 1 Trial to be safe and deplete cardiac ATTR in a dose dependent manner (Garcia-Pavia *et al.*, N Engl J Med (2023), doi: 10.1056/NEJMoa2303765. Overviews of the different treatment strategies are given in Tomasoni *et al.*, Front Cardiovasc Med 10 (2023), 1154594.

Though each of the current drugs and concepts holds great promise to ameliorate ATTR and conditions associated therewith, alternative therapeutic strategies are still needed since every patient reacts differently to a certain treatment and thus, a selection of different treatment options is always favorable.

This technical problem is solved by the embodiments characterized in the claims and described further below and illustrated in the Examples and Figures.

SUMMARY OF THE INVENTION

The present invention relates generally to vaccines useful in the treatment and prevention of amyloid transthyretin amyloidosis (ATTR) and disorders caused by or associated with TTR deposits. While TTR tetramer stabilizers, gene silencers, or antibodies are predominantly used in the treatment of acute and chronic disorders caused by ATTR, *i.e.*, at the onset or presence of the disease, vaccines can be used before the onset of the disease for the prevention of the disease in subjects at risk for example because of hereditary predisposition or determined to be at risk, for example by the alteration of the level of indicative biomarkers. In addition, there is usually no need for continued administration of the therapeutic agent since vaccination results in perpetual protection due to the priming of the immune system. More specifically, the present invention relates to a vaccine comprising an immunogen, which is in the context of the present invention a peptide derived from TTR, *i.e.*, a TTR peptide, which comprises an epitope of TTR, the epitope being selectively presented or accessible only in the misfolded, oligomeric, and/or aggregated form of TTR, as in the case of a neoepitope, and/or the epitope being at least not present in the physiologically active form of TTR, *e.g.*, in the case of an epitope accessible in the monomer of the wildtype or mutant TTR protein, which is hidden in the physiologically active tetramer and is no longer accessible to antibody binding. Most preferably, the epitope is not present on native TTR monomers to avoid any cross-reactivity. Typically, the peptide-based vaccine of the present invention comprises the TTR peptide and an adjuvant to stimulate an immune response. The vaccine of the present invention is preferably used in the treatment and prevention of ATTR.

About two decades ago, Terazaki *et al.*, Lab Invest. 86 (2006) 23-31, described immunization of transgenic mice carrying the most common FAP-associated TTR mutant - V30M (transthyretin mutant with methionine replacing valine at position 30) with a TTR mutant - Y78F (transthyretin mutant with phenylalanine replacing tyrosine at position 78) - designed to destabilize the native structure, which has been shown to expose a cryptic epitope recognized by a monoclonal antibody that reacts only with highly amyloidogenic mutants presenting the amyloid fold or with amyloid fibrils. Their results suggested that Y78F induced production of an antibody that reacts specifically with deposits and leads to an immune response effective in removing/preventing TTR deposition. Therefore, the authors concluded that TTR immunization with selected TTR mutants could have potential application in immune therapy for FAP.

However, since then this approach has not been further pursued for establishing a vaccine, probably because, as such, one significant concern that is associated with active immunotherapy

is that the antigen, here mutant TTR protein nevertheless may invoke an immune response to physiological TTR species. On the other hand, the Y78F mutant protein has been used because it was thought that by using this mutant TTR protein the cryptic epitopes can be displayed to provoke the desired immune response.

The present invention is based on the concept of identifying segments in the TTR amino acid sequence which are accessible to the body's immune response but unique for TTR fibrils and amyloid and designing a corresponding peptide comprising at least an immunogenic part of that region and forcing the peptide and immunogenic part, respectively, into a 3D conformation that resembles the one the respective segment may have in the amyloid conformation. For this purpose, based on the possible mechanism of misfolding of TTR protein, Cryo-EM studies have been screened that suggest that after the disassembly and unfolding of the native tetramer, followed by the assembly of the polypeptide chains into an early fibril state, a structurally disordered segment of residues Ala36-His56 in a solvent exposed conformation is formed, which is not present on native TTR tetramer; see Schmidt *et al.*, Nat Commun 10 (2019), 5008, especially Figure 3 and its Figure legend which are incorporated herein by reference. Indeed, recent publications reported the 3D structure of ATTR fibrils extracted from patient tissues and determined using cryogenic electronic microscopy (cryo-EM); see Schmidt *et al.* (2019), *supra*; Iakovleva *et al.*, Nat Commun 12 (2021), 7141; Steinebrei *et al.*, Nat Commun 13 (2022), 6398.

The amyloid structure was virtually identical in all three cases and characterized by a rigid core structure interrupted by an unresolved segment going from Lys35 to Gly57. This indicated the presence of a loose segment with a variable, non-rigid conformation. Nevertheless, in accordance with the present invention, the proximity of Lys35 and Gly57 in the amyloid core indicates that the unresolved segment formed a loop, which as illustrated in the Examples has been used to design corresponding peptides.

Inspection of the amino acid sequence revealed that the loose segment comprises the epitope of NI006, *i.e.*, WEPFA (SEQ ID NO: 1), *i.e.*, the selective anti-ATTR antibody that has been shown to remove cardiac amyloid; see Garcia-Pavia *et al.* (2023), *supra*, and Michalon *et al.*, Nat Commun 12 (2021), 3142. Accordingly, it is prudent to assume that NI006 binds to the loose segment in ATTR and thus could be used for determining if a peptide derived from that segment could be designed such that the 3D conformation in the TTR amyloid could be

mimicked and the antibody substantially binds as selective and with high affinity as to the TTR amyloid.

In this context, it has to be taken into account that the peptide, *i.e.*, immunogen is typically formulated with an adjuvant or an immunogenicity enhancing agent, like a protein carrier to produce an immune response strong enough to protect a subject from the disease he or she is being vaccinated against, and that the presence of for example the protein carrier could have a negative impact on the structure of the peptide and result in loss of the formation of the neoepitope as found in the 3D conformation of the loose segment in TTR amyloid.

Therefore, the desired 3D conformation of the peptide has been tried to stabilize by triggering the cyclization of the corresponding peptide sequence.

Hence, experiments performed within the scope of the present invention surprisingly revealed that TTR peptides coupled to Bovine Serum Albumin (BSA) as a carrier can serve as antigens for an anti-TTR antibody, and that cyclization of said peptides promotes antibody binding by more than 100-fold; see Examples 1 and 2. In particular, anti-TTR antibody binding, shown exemplarily based on antibody NI-301.37F1, to the linear TTR peptide TTR34-54 and to the cyclic peptide TTR34-54cyc, which both comprise the epitope WEPFA (SEQ ID NO: 1), in the nanomolar and picomolar range, respectively, was observed in ELISA assays; see Example 1. Furthermore, coupling of a carrier protein, here exemplarily shown with BSA, to TTR peptides, in particular to the cyclic peptides TTR34-54cyc and TTR39-50cyc, still enables antibody binding; see Example 2.

Antibody NI-301.37F1, which binds the TTR epitope WEPFA (SEQ ID NO: 1) is described in WO 2015/092077 A1. In addition, the utility of the TTR peptides as an immunogen and thus for a vaccination approach was confirmed *in vivo* mouse studies as described in Example 3. In particular, mice were immunized with linear and cyclic BSA-coupled TTR peptides of 12 and 21 amino-acid length and the immune response was characterized by ELISA against the amyloid TTR (ATTR, mis.WT-TTR) and tetrameric TTR (TTR). As shown in Fig. 3, the administration of the TTR peptides elicited the formation of antibodies against TTR and ATTR after 38 days, wherein the tested sera exhibited a much stronger serum reactivity towards ATTR than towards TTR. Surprisingly, and in contrast what could have been expected from the initial ELISA assays described in Example 1 and 2, both linear peptides TTR34-54 and TTR39-50

exhibited a higher amyloid selectivity with an $EC_{50(ATTR/TTR)}$ quotient of 15.8 and 14.7, respectively, than the cyclic peptide TTR34-54cyc showing an amyloid selectivity with an $EC_{50(ATTR/TTR)}$ quotient of 13.4 and the cyclic peptide TTR39-50cyc showing an amyloid selectivity with an $EC_{50(ATTR/TTR)}$ quotient of 4.3, see Table 1 and Fig. 3. Furthermore, the highest titer of ATTR-specific antibodies was detected in serum obtained from mice that have been immunized with the linear peptide TTR34-54, *i.e.*, the mice to which the TTR34-54 immunogen was administered showed a higher immune response in comparison to the mice to which the other peptides were administered; see Fig. 4.

Accordingly, further evidence could be provided that since the exemplarily tested TTR peptides seem to mimic the 3D conformation of the loose segment in TTR amyloid and shall raise neoepitope specific immune response in a mammal, it is prudent to expect that TTR peptides designed in accordance with the present invention, when administered to a subject, for example in the form of a vaccine, trigger an immune response, including a stimulation of T-cells and other reactive immune cells directed against the TTR peptide, and lead to the generation of anti-TTR antibodies presenting a high selectivity for amyloidogenic TTR. As explained above, antibodies binding within segment of TTR ranging from Lys35 to Gly57 have the capability to clear misfolded TTR aggregates by phagocytosis and thus, it is prudent to expect that TTR peptides specifically eliciting antibodies against that segment, preferably including the amino acid sequence WEPFA (SEQ ID NO: 1), can be used as immunogens in vaccines and induce the clearance of misfolded TTR aggregates before they can further assemble to fibrils. This will avoid the manifestation of the disease phenotype, *i.e.*, the accumulation of TTR fibrils. Accordingly, it is prudent to expect that the TTR peptide of the present invention can be used for immunization, *i.e.*, to initiate an immune response against amyloidogenic/misfolded TTR in order to prevent the onset of ATTR or in case that the disease has manifested itself already in the treatment of said disease. The theory underlying the present invention is confirmed by Example 3 demonstrating an effective immune response against immunogens of the present invention, wherein the serum antibodies preferentially bind to aggregated TTR. Preventing the (further) formation of TTR aggregates is believed to prevent further aggravation of the disease and/or complete remission of the TTR aggregates and may stop the disease cascade.

As mentioned, the structurally disordered segment of residues Ala36-His56 of TTR, that is formed in a solvent exposed conformation in TTR amyloid and not present on native TTR tetramer, *i.e.*, being a neoepitope is the preferred basis for the design of the TTR peptide.

Binding of antibodies to such neoepitopes is the key for their safety and their therapeutic efficacy and thus, the elicitation of such antibodies by an immunogen is the key for the effectiveness of the vaccine comprising said immunogen. Accordingly, the present invention relates to a vaccine comprising a peptide derived from TTR which comprises an epitope of TTR, the epitope being selectively presented or accessible to binding by an antibody only in the misfolded, oligomeric, and/or aggregated form of TTR. In a preferred embodiment, the vaccine of the present invention comprises a peptide, which comprises the amino acid sequence WEPFA (SEQ ID NO: 1), and which preferably comprises or consists of the amino acid sequence RKAADDTWEPFASGKTSESGE (SEQ ID NO: 2, TTR34-54) or DTWEPFASGKTS (SEQ ID NO: 3, TTR39-50), most preferably the amino acid sequence RKAADDTWEPFASGKTSESGE (SEQ ID NO: 2, TTR34-54). In a preferred embodiment, the peptide is a linear peptide.

Subunit vaccines that consist primarily of peptides or proteins can face limitations with respect to immunogenicity and thus may require multiple immunizations to achieve high levels of immune response, which led to the development of a variety of approaches to enhance subunit vaccine responses, including presentation of epitopes in multimeric format (*e.g.*, virus-like particles, VLPs, or nanoparticles). This strategy can boost immune responses by increasing the half-life of the epitope by decreasing renal clearance and susceptibility to proteolytic degradation; see Malonis *et al.*, Chem Rev. 120 (2020), 3210–3229. Without intending to be bound by theory, the use of a cyclic peptide as vaccine could have a similar effect, *i.e.*, to enhance vaccine response, due to a very stable conformation adopted by the cyclic peptide since it is constrained by having the two extremities connected together. Thus, in one embodiment, the vaccine of the present invention comprises a TTR peptide capable of forming a cyclic compound, *i.e.*, the vaccine of the present invention comprises in one embodiment a cyclized TTR compound. Cyclization is preferably performed by a linker which is coupled to the peptide N-terminus residue and the C-terminus residue. In a preferred embodiment, the cyclic compound comprises or consists of the amino acid sequence GCGGGRKAADDTWEPFASGKTSESGEGGGCG (SEQ ID NO: 4, TTR34-54cyc), or GCGGGDTWEPFASGKTSGGGCG (SEQ ID NO: 5, TTR39-50cyc).

The observation made in Example 2 that BSA coupling does not substantially interfere with antibody binding is important for the successful development of a peptide-based vaccine. In particular, in typical peptide vaccination protocols, in addition or alternatively to the approach

to present epitopes in a multimeric format, the peptide comprising the epitope of interest can be conjugated to a carrier protein which can boost immune responses by increasing the half-life of the epitope by decreasing renal clearance and susceptibility to proteolytic degradation. Linkage to carrier proteins is typically achieved by chemical conjugation. The carriers are generally known to have immunogenic properties, and thus the simple covalent linking of epitopes to immunogenic species can often be sufficient to enhance the immune response; see Malonis *et al.*, Chem Rev. 120 (2020), 3210–3229. BSA is an example of an immunogenic carrier. Thus, the TTR peptides seem to be suitable candidates for a vaccine. Accordingly, in one embodiment, the vaccine of the present invention comprises a peptide, preferably in cyclized form, which is coupled to carrier protein, for example BSA.

As discussed hereinbefore, presentation of the correct 3D structure of the neoepitope is key to elicit an immune response which preferentially recognizes ATTR, *i.e.*, aggregated TTR, especially of wildtype TTR species. Therefore, it has been initially thought that, besides conferring serum stability, cyclization of the peptide could aid to the folding of the peptide to mimic the structure on misfolded and aggregated TTR and stabilize the conformation against potential detrimental inference with correct folding by the immunogenic carrier, here BSA which is a much larger polypeptide and could well have been thought to have a negative effect on folding of the peptide or mask the epitope. However, experiments performed in accordance with the present invention surprisingly revealed that cyclization is neither needed nor advantageous for the efficacy and specificity of the TTR peptide immunogen; see Example 3, and Fig. 3 and 4.

Accordingly, in one preferred embodiment of the present invention, the TTR peptide-based immunogen is not cyclized and preferably also not modified otherwise. Rather, the TTR peptide in the immunogen preferably comprises the minimal epitope/antigenic sequence determined experimentally and/or by *in silico* analysis to represent a neoepitope specific of the pathological variant, oligomer and/or aggregate of TTR, and optionally in addition about 1 to 10 amino acids at its N- and/or C-terminus, preferably wherein the additional amino acids are also present in the original TTR amino acid sequence; see also the Examples.

As mentioned above, the immunogen for the vaccine of the present invention can be synthesized chemically or produced using recombinant DNA techniques; see for review, *e.g.*, Wang *et al.*, Sig Transduct. Target Ther. 7 (2022); https://doi.org/10.1038/s41392-022-00904-

4. For any recombinantly expressed antigenic TTR peptide according to the present invention coupled or not to an immunogenic carrier, the nucleic acid which encodes said peptide or protein also forms an aspect of the present invention, as does an expression vector comprising the nucleic acid, and a host cell containing the expression vector (autonomously or chromosomally inserted).

Accordingly, in one or more embodiments, the present invention pertains to a nucleic acid encoding the immunogen as herein described; an expression vector comprising such nucleic acid; and/or a host cell comprising the nucleic acid or the expression vector. A method of recombinantly producing the immunogen by expressing it in a host cell and isolating the immunogen therefrom is a further aspect of the invention.

The nucleic acid and expression vector of the present invention may be part of a kit or composition, which optionally further comprises an immunogenicity enhancing agent, like an immunogenic carrier, or an adjuvant

The present invention further relates to a kit comprising the vaccine of the present invention.

Further embodiments of the present invention will be apparent from the description and Examples that follow.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1: Results of ELISA assays show that exemplarily tested TTR peptides retain 3D conformation of the loose segment uniquely present in TTR amyloid by binding to ATTR specific antibody NI-301.37F1 and that cyclization promotes antibody binding, *i.e.*, TTR peptides, in particular cyclic TTR peptides are suitable as antigens and immunogens; **(A)** in ELISA-1, antibody binding to the peptide TTR34-54cyc and mis-WT-TTR was analyzed and the results showed that antibody binding to the cyclic TTR34-54cyc peptide is much stronger, *i.e.*, about 10-fold stronger, than binding to mis.WT-TTR; **(B)** in ELISA-2, antibody binding to the peptides TTR34-54_bt and TTR34-54cyc_bt as well as to a BSA control was analyzed and the results showed that that both linear as well as cyclic TTR peptides were bound by the ATTR specific antibody, but that cyclization promotes NI-301.37F1 binding by more than 100-fold; **(C)** in ELISA-3, antibody binding to the peptides TTR34-54cyc and TTR40-49 was

analyzed and no antibody binding to TTR40-49 was observed, indicating that a certain length of the peptide and antigenic/immunogenic part of the peptide, respectively, is required for proper folding.

Fig. 2: Results of ELISA assays showed that BSA-coupled TTR peptides display TTR amyloid specific epitope as demonstrated by binding to antibody NI-301.37F1; **(A)** in ELISA-1, antibody binding to the peptides TTR34-54cyc_BSA, TTR34-54_BSA, TTR34-54SCRcyc_BSA, and TTR34-54SCR_BSA was analyzed and the results showed that both the linear as well as the cyclic TTR peptides coupled to BSA were bound by the antibody, but that cyclization promotes NI-301.37F1 binding by more than 100-fold; **(B)** in ELISA-2, antibody binding to the peptides TTR39-50cyc_BSA, TTR39-50_BSA, TTR39-50SCRcyc_BSA, and TTR39-50SCR_BSA was analyzed and the results showed antibody binding to the cyclic BSA coupled TTR39-50cyc_BSA peptide, but that binding to the corresponding linear peptide could hardly be detected. No binding to the antigen controls, *i.e.,* to the scrambled (SCR) peptides, was observed in both ELISA assays.

Fig. 3: Results of ELISA assays showed that the immune response as measured by serum antibody titers increased over time (0 days (d0) compared to 38 days (d38)) and that the reactivity of serum obtained from BalbC mice after immunization with TTR peptides towards ATTR was higher than towards TTR; (**A**) ELISA against TTR and ATTR showed a reactivity (EC_{50}) of serum obtained from mice after immunization with the peptide/immunogen TTR39-50 towards TTR of 1038 and towards ATTR of 15271 and thus, an amyloid selectivity ($EC_{50(ATTR/TTR)}$ quotient) of 14.7; (**B**) ELISA against TTR and ATTR showed a reactivity (EC_{50}) of serum obtained from mice after immunization with the peptide/immunogen TTR39-50cyc towards TTR of 1813 and towards ATTR of 24343 and thus, an amyloid selectivity ($EC_{50(ATTR/TTR)}$ quotient) of 15.4; (**C**) ELISA against TTR and ATTR showed a reactivity (EC_{50}) of serum obtained from mice after immunization with the peptide/immunogen TTR34-54 towards TTR of 3400 and towards ATTR of 53809 and thus, an amyloid selectivity ($EC_{50(ATTR/TTR)}$ quotient) of 15.8; (**D**) ELISA against TTR and ATTR showed a reactivity (EC_{50}) of serum obtained from mice after immunization with the peptide/immunogen TTR34-54cyc towards TTR of 7785 and towards ATTR of 33587 and thus, an amyloid selectivity ($EC_{50(ATTR/TTR)}$ quotient) of 4.3; (**D**) control ELISA against TTR and ATTR

showed a reactivity (EC_{50}) of serum obtained from mice after immunization with a control peptide/immunogen PR906. The OD_{450} values of the sera were measured and plotted against the serum dilution (dilution range from 1:100 to 1:5,904,900) on a logarithmic scale.

Fig. 4: Results of ELISA assays showed that the reactivity of serum obtained from BalbC mice after immunization with the TTR peptides TTR39-50, TTR39-50cyc, TTR34-54, and TTR34-54cyc towards ATTR was higher than towards TTR and that the highest antibody titer, in particular titer of ATTR-specific antibodies, was detected in serum obtained from mice that have been immunized with the linear peptide TTR34-54. For the assay, a serum dilution of 1:24,300 has been used.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a peptide-based vaccine comprising a peptide derived from transthyretin (TTR), *i.e.*, a TTR peptide, which comprises an epitope of TTR, the epitope being selectively presented or accessible to binding by an antibody only in the misfolded, oligomeric, and/or aggregated form of TTR.

Unless defined otherwise in the present application, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the exemplary methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples are illustrative only and not intended to be limiting.

Further embodiments of the present invention will be apparent from the description, Examples and claims that follow. The person skilled in the art will appreciate that every characterization of a generic feature of a general embodiment in the following can and preferably are intended to be combined with the characterization of one or more of the other features of such general embodiment. For the avoidance of any doubt it is emphasized that the expressions "in some embodiments", "in a certain embodiments", "in certain instances", "in some instances", "in a further embodiment", "in one embodiment" and the like are used and meant such that any of the embodiments described therein are to be read with a mind to combine each of the features

of those embodiments and that the disclosure has to be treated in the same way as if the combination of the features of those embodiments would be spelled out in one embodiment. The same is true for any combination of embodiments and features of the appended claims and illustrated in the Examples, which are also intended to be combined with features from corresponding embodiments disclosed in the description, wherein only for the sake of consistency and conciseness the embodiments are characterized by dependencies while in fact each embodiment and combination of features, which could be construed due to the (multiple) dependencies must be seen to be literally disclosed and not considered as a selection among different choices.

Unless otherwise stated, a term as used herein is given the definition as provided in the Oxford Dictionary of Biochemistry and Molecular Biology, Oxford University Press, 1997, revised 2000 and reprinted 2003, ISBN 0 19 850673 2; Second edition published 2006, ISBN 0-19-852917-1 978-0-19852917-0.

Reference to the "cyclic peptide" herein can refer to a fully proteinaceous compound, *e.g.*, wherein the linker is 2, 3, 4, 5, 6, 7 or 8 amino acids, or wherein no linker is present. For example, it is possible that the native protein sequence, *i.e.*, amino acid stretch comprising the epitope of the antibody allows cyclization, for example due to the presence of two cysteines in appropriate distance, without addition of extra amino acids. It is understood that properties described for the cyclic peptide determined in the examples can be incorporated in other compounds, *e.g.*, cyclic compounds comprising non-amino acid linker molecules. "Cyclic peptide" and "cyclic compound" can be used interchangeably when the cyclic compound is composed of amino acids.

The term "immunogenic" refers to substances which elicit the production of antibodies, activate lymphocytes or other reactive immune cells directed against an antigenic portion of the immunogen, in the present case the TTR peptides.

An "immunogen" or "immunogenic compound" as used herein means a substance which provokes an immune response and causes production of an antibody and can comprise for example the TTR peptides, in particular the cyclic peptides described herein, conjugated as multiantigenic peptide and/or fused to an immunogenicity enhancing agent such as a carrier protein, like BSA. In addition to the conjugates described herein, immunogenic peptide

mimetics which elicit cross-reactive antibodies to the epitopes mentioned herein constitute immunogens.

The term "corresponding linear compound" with regard to a cyclic compound refers to a compound, optionally a peptide, comprising or consisting of the same sequence or chemical moieties as the cyclic compound but in linear (non-cyclized) form.

The term "linker" as used herein means a chemical moiety, preferably poorly immunogenic or non-immunogenic, that can be covalently linked directly or indirectly to the protein fragment or peptide as defined herein. The linker ends can for example be joined to produce a cyclic compound. The linker can be present at a location at the N- and C-termini. Alternatively, the linker may at an internal position at "some distance" from the termini. The linker ends can for example be joined to produce a cyclic compound. The linker can comprise one or more functionalizable moieties such as one or more cysteine (C) residues. The linker can be also linked via the functionalizable moieties to other proteins or components. The linker can be linked via the functionalizable moieties to a carrier protein or an immunogen enhancing agent such as keyhole limpet hemocyanin (KLH) or bovine serum albumin (BSA). The cyclic compound comprising the linker is of longer length than the peptide or protein fragment itself. The linker may include, but is not limited to, non-immunogenic moieties such as amino acids Glycine (G), and Alanine (A), or polyethylene glycol (PEG) repeats.

The term "functionalizable moiety" as used herein refers to a chemical entity with a "functional group" which as used herein refers to a group of atoms or a single atom that will react with another group of atoms or a single atom (so called "complementary functional group") to form a chemical interaction between the two groups or atoms. In the case of cysteine (C), the functional group can be -SH which can be reacted to form a disulfide bond. The reaction with another group of atoms can be covalent or a strong non-covalent bond, for example as in the case as biotin-streptavidin bonds, which can have dissociation constant (Kd) of about 1e-14. A strong non-covalent bond as used herein means an interaction with a Kd of at least 1 e-9, at least 1e-10, at least 1 e-11, at least 1 e-12, at least 1 e-13 or at least 1 e-14.

Proteins and/or other agents may be coupled to the cyclic compound, for example to aid in immunogenicity. For this purpose, any functionalizable moiety capable of reacting (*e.g.*, making a covalent or non-covalent but strong bond) may be used. In one specific embodiment,

the functionalizable moiety is a cysteine residue which is reacted to form a disulfide bond with an unpaired cysteine on a protein of interest, which can be, for example, an immunogenicity enhancing agent such as a carrier protein like Bovine serum albumin (BSA), or a T helper cell epitope. The term "reacts with" as used herein generally means that there is a flow of electrons or a transfer of electrostatic charge resulting in the formation of a chemical interaction.

As used herein, the phrase "effective amount" refers to the amount of antigenic/immunogenic composition which, when administered to a human or animal, elicits an immune response. The effective amount is readily determined by one of skill in the art following routine procedures.

The term "carrier protein" as used herein refers to an immunogenic protein that is used to increase the response of the immune system to an otherwise not sufficiently immunogenic compound, such as another protein/peptide. Carrier proteins are in particular useful for small compounds that have a very limited number of possible epitopes on which an immune response can be directed or compounds that have a very low immunogenicity. Preferred carrier proteins have a high compound to carrier ratio by allowing the coupling of numerous compounds to a single carrier protein in order to increase the immunogenicity of a carrier protein antigen complex.

The term "adjuvant" refers to compounds that, when administered in conjunction with an antigen, here the TTR peptide, augment, stimulate, activate, potentiate, or modulate the immune response to the antigen, but when administered alone does not generate an immune response to the antigen. Adjuvants can augment an immune response by several mechanisms including lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages. An adjuvant may be a natural compound, a modified version of or derivative of a natural compound, or a synthetic compound. Examples of such adjuvants include but are not limited to inorganic adjuvants (*e.g.*, inorganic metal salts such as aluminum phosphate or aluminum hydroxide such as Alhydrogel® often referred to as alum), organic adjuvants (*e.g.*, saponins or squalene), oil-based adjuvants (*e.g.*, Freund's complete adjuvant and Freund's incomplete adjuvant, cytokines (*e.g.*, IL-IB, IL-2, IL-7, IL-12, IL-18, GM-CFS, and INF-y) particulate adjuvants (*e.g.*, immuno-stimulatory complexes (ISCOMS), liposomes, or biodegradable microspheres), virosomes, bacterial adjuvants (*e.g.*, monophosphoryl lipid A (MPL), or muramyl peptides), synthetic adjuvants (*e.g.*, non-ionic block copolymers, muramyl peptide analogues, or synthetic lipid A), or polynucleotides adjuvants (*e.g.*, CpG oligodeoxynucleotides. QS-21 is a saponin extracted from *Quillaja saponaria*. MF59 is an oil-

in water emulsion comprising squalene, polysorbate 80 and sorbitantrioleat. AS03 is an oil-in-water emulsion comprising squalene, polysorbate 80 and a-tocopherol. AS0l is a combination of liposomes, MPL and QS21. AS02 is a combination of oil-in-water emulsion, MPL and QS21. AS04 is a complex of MPL and aluminum hydroxide or aluminum phosphate. IC3l is a combination of a KLK peptide with the oligodesoxynucleotide ODNI. Hiltonol (Poly-ICLC) is a synthetic complex of carboxymethylcellulose, polyinosinic-polycytidylic acid, and poly-L-lysine double-stranded RNA.

The term "immunization" refers to the process of activating, strengthening or boosting the immune system of an individual against an agent ("vaccine") which typically causes or induces a disease or disorder. Hence, by immunizing a healthy individual against said agent, the onset of a disease or disorder may be prevented. Immunizing a patient suffering from a disease or disorder may treat said disease or disorder or may prevent the further progression of said disease. Immunization may be achieved through various techniques, most commonly immunization is achieved through vaccination of the healthy individual or the patient suffering from a disease or disorder. The principle underlying active immunization via a vaccine, here comprising a TTR peptide, is the generation of an immunological "memory". In the context of ATTR, where the cellular antigen, *i.e.*, amyloidogenic TTR is continuously present, the aim of active immunization is to maintain high antibody levels continuously by life-long, regular immunization. Challenging an individual's immune system with *e.g.*, a vaccine comprising a disease specific immunogen, induces the formation and/or propagation of immune cells which specifically recognize the immunogen comprised by the vaccine. At least a part of said immune cells remains viable for a period of time which can extend to 10, 20 or 30 years after vaccination. If the individual's immune system encounters the immunogen again within the aforementioned period of time, the immune cells generated by vaccination are reactivated and enhance the immune response against the immunogen as compared to the immune response of an individual which has not been challenged with the vaccine and encounters said immunogen for the first time. In many cases, a single administration of a vaccine is not sufficient to generate the number of long-lasting immune cells which is required for effective protection against said diseases or disorder. Consequently, repeated challenge with a biological preparation specific for a specific disease is required in order to establish lasting and protective immunity against said disease or to cure a given disease.

As explained in detail above, the present invention is based on the concept of identifying segments in the TTR amino acid sequence which are accessible to the body's immune response but unique for TTR fibrils and amyloid, and inspection of the amino acid sequence revealed that such segment comprises the epitope of NI006, *i.e.*, WEPFA (SEQ ID NO: 1). As illustrated in the Examples, different TTR peptides that comprise the epitope WEPFA (SEQ ID NO: 1) which is comprised in the loose segment and which is a neoepitope in the sense that is hidden in the TTR protein's naturally folded conformation but accessible to antibody binding following unfolding and aggregation, maintain the amyloid specific conformation and thus are suitable as an immunogen. As explained in more detail above, it is prudent to expect that such peptides, once administered to the subject, *e.g.*, in a form of a vaccine, trigger an immune response specific to TTR aggregates resulting in TTR amyloid clearance as observed for the passive immune therapy with NI006 and therefore are also able to prevent the manifestation of ATTR. Thus, the TTR peptides described herein are thus useful as immunogens in vaccines.

Accordingly, the present invention relates to a vaccine comprising a peptide derived from TTR, *i.e.*, a TTR peptide, which comprises an epitope of TTR, the epitope being selectively presented or accessible only in the misfolded, oligomeric, and/or aggregated form of TTR as well as to the use of said TTR peptide as immunogen, *i.e.*, as immunogenic compound. In other words, the present invention relates to a peptide derived from TTR *i.e.*, a TTR peptide, which comprises an epitope of TTR, the epitope being selectively presented or accessible only in the misfolded, oligomeric, and/or aggregated form of TTR, for use as a vaccine.

In a particular preferred embodiment, the TTR peptide is derived from the loose segment of TTR ranging from Lys35 to Gly57. Thus, it is prudent to expect that further TTR peptides comprising epitopes being present in or overlap with said loose fragment and being exposed in the misfolded variant, and on aggregates, fibrils and/or oligomers of TTR, respectively, are suitable for the vaccine of the present invention. Thus, in one embodiment, the TTR peptide comprised in the vaccine of the present invention comprises at least 4 amino acid residues and preferably all amino acids of an amino acid sequence being present in or overlap with said loose TTR fragment and being exposed in the misfolded variant, and on aggregates, fibrils and/or oligomers of TTR, respectively, *e.g.*, 54-ELXGLTXE-61 (SEQ ID NO: 13), which is a peptide recognized for example by antibody NI-301.35G11 disclosed in WO 2015/092077 A1, wherein X can be any amino acid; WEPFASG (SEQ ID NO: 14), which is a peptide recognized for example by antibody NI-301.12D3 disclosed in WO 2015/092077 A1; and 30-

VHVFRKAADDTWEPFASGKTSESGELHGLTTEEEFVE-66 (SEQ ID NO: 20), which is a peptide recognized for example by an antibody described in WO 2014/124334 A2. Furthermore, the amino acid sequence used in the TTR peptide may be altered, for example by amino acid substitutions/deletions/additions compared to the original amino acid sequence in the loose segment as long as the 3D conformation remains unaffected in kind. For example, the TTR peptide may comprise an epitope comprising the amino acid sequence WXPFA (SEQ ID NO: 11), which is a peptide recognized for example by antibody NI-301.28B3 disclosed in WO 2015/092077 A1, wherein X can be any naturally occurring amino acid.

It is further prudent to expect that also further TTR peptides comprising epitopes that are exposed in the misfolded variant, and on aggregates, fibrils and/or oligomers, respectively, of TTR, are in general able to promote the elicitation of antibodies able to clear pathological TTR aggregates when administered to a subject and are thus useful as immunogens in vaccines. Thus, in one embodiment, the TTR peptide comprised in the vaccine of the present invention comprises at least 4 amino acid residues and preferably all amino acids of an amino acid sequence which is exposed in the misfolded variant, and on aggregates, fibrils and/or oligomers of TTR, respectively, *e.g.*, EEFXEGIY (SEQ ID NO: 12), which is a peptide recognized for example by antibody NI-301.59F1 disclosed in WO 2015/092077 A1, wherein X can be any amino acid; TTAVVTNPKE (SEQ ID NO: 15), which is a peptide recognized for example by antibody NI-301.18C4 disclosed in WO 2015/092077 A1; KCPLMVK and VFRK (SEQ ID NOs: 16 and 17), which represent peptides comprising a conformational epitope requiring at least the C of the first sequence and the V and F of the second sequence, and which is an epitope recognized by antibody NI-301.44E4 of WO 2015/092077 A1; EHAEVVFTA (SEQ ID NO: 18), which is a peptide recognized for example by antibody 14G8 / PRX004 / NN-6019 disclosed *inter alia* in Higaki *et al.*, Amyloid 23 (2016), 86-97; GPRRYTIAA (SEQ ID NO: 19), which is a peptide recognized for example by antibody 18C5 described in WO 2019/071205 A1; ALLSPYSYSTTAV (SEQ ID NO: 21), which is a peptide recognized for example by an antibody described in WO 2014/124334 A2 which binds to TTR109-121; WKALGISPFHE (SEQ ID NO: 22), which is a peptide recognized for example by antibody 371M described in WO 2015/115332 A1; SYSTTAVVTN (SEQ ID NO: 23), which is a peptide recognized for example by antibody 313M (RT24) described in WO 2015/115331 A1; or LLSPYSYSTTAVVTNPKE (SEQ ID NO: 24), which is a peptide recognized for example by an antibody described in WO 2014/124334 A2 which binds to TTR100-127.

The amino acid sequence comprised in the loose segment of TTR is derived from the wild type TTR amino acid sequence and not specific to any variant TTR (TTRv). Accordingly, in one embodiment, the TTR peptide comprised in the vaccine of the present invention comprises an epitope consisting of a wild type TTR amino acid sequence and thus, the vaccine of the present invention is useful in preventing the occurrence of sporadic, wild-type-ATTR, which is triggered by misfolded wild type TTR.

As mentioned above, the exemplarily TTR epitope WEPFA (SEQ ID NO: 1) is a neoepitope which is not only selectively presented and accessible, respectively, in misfolded, oligomeric and/or aggregated forms of TTR, but it is also not present in the physiologically active tetrameric form of TTR, and also not present on native TTR monomers. Thus, in accordance with the present invention, the TTR peptide comprises an epitope which is selectively presented or accessible in misfolded, oligomeric and/or aggregated forms of TTR. In addition, or alternatively, the TTR peptide comprises an epitope which is not present in the physiologically active tetrameric form of TTR (but might be present on native and/or mutant TTR monomers), but preferably, the epitope is also not present on native TTR monomers. Further exemplarily epitopes that are not present on native TTR monomers, are known from applicant's previous work disclosed in WO 2015/092077 A1 and comprise for example the amino acid sequence EEFXEGIY (SEQ ID NO: 12), which is an epitope recognized for example by antibody NI-301.59F1, wherein X can be any amino acid, or ELXGLTXE (SEQ ID NO: 13), which is a peptide recognized for example by antibody NI-301.35G11, wherein X can be any amino acid. Accordingly, in one embodiment, the TTR peptide comprised in the vaccine of the present invention comprises an epitope comprising or consisting of the amino acid sequences WEPFA (SEQ ID NO: 1), EEFXEGIY (SEQ ID NO: 12), or ELXGLTXE (SEQ ID NO: 13). Most preferably, the TTR peptide comprises the amino acid sequence WEPFA (SEQ ID NO: 1).

As further shown in the Examples, in particular in Example 2, the tested anti-TTR antibody was shown to bind to TTR34-54 as well as to TTR39-50.

Thus, in one embodiment of the present invention, the TTR peptide comprises or consists of between 5 and 40 amino acids, preferably between 10 and 30 amino acids, more preferably between 12 and 25 amino acids, more preferably between 12 and 21 amino acids, more preferably 12 or 21 amino acids, and most preferably 21 amino acids of the TTR protein. In one embodiment, the TTR peptide comprises or consists of at least 5 amino acids, preferably at least

10, more preferably at least 12 amino acids, more preferably at least 15, most preferably at least 20, 21, 22, 23, 24, or 25 amino acid residues of the TTR protein. As mentioned above, these amino acid residues comprise an epitope presented or accessible in misfolded, oligomeric and/or aggregated forms of TTR. More specifically, at least the epitope, which as known to the person skilled in the art can consist of as few amino acids as four should be present, which may be supplemented with appropriate number of amino acids and/or other moieties sufficient and necessary for providing a stable peptide, for example linker moieties necessary for cyclization.

However, in principle there is no limitation as to length of the peptide as long as it is stable and immunogenic, *i.e.*, elicits antibodies once administered to a subject, and optionally can be cyclized. Accordingly, the TTR peptide comprised in the vaccine of the present invention contains between 4 amino acids and all amino acids of the TTR protein. Preferably, the TTR peptide comprises between 4 amino acids and 100 amino acids, more preferably between 4 amino acids and 90 amino acids, more preferably between 4 amino acids and 80 amino acids, more preferably between 4 amino acids and 70 amino acids, more preferably between 4 amino acids and 60 amino acids, more preferably between 4 amino acids and 50 amino acids, more preferably between 4 amino acids and 45 amino acids, more preferably between 4 amino acids and 40 amino acids, more preferably between 4 amino acids and 35 amino acids, more preferably between 4 amino acids and 30 amino acids, more preferably between 4 amino acids and 25 amino acids, or between 4 amino acids and 24 amino acids, or between 4 amino acids and 23 amino acids, or between 4 amino acids and 22 amino acids, or between 4 amino acids and 21 amino acids, or between 4 amino acids and 20 amino acids, preferably between 5 amino acids and 25 amino acids, or between 5 amino acids and 24 amino acids, or between 5 amino acids and 23 amino acids, or between 5 amino acids and 22 amino acids, or between 5 amino acids and 21 amino acids, or between 5 amino acids and 20 amino acids.

The amino acids either represent only the epitope or the epitope and adjacent amino acids present in the TTR protein. In a preferred embodiment, the TTR peptide comprises amino acid residues of the TTR protein, wherein these amino acid residues comprise the epitope and adjacent amino acids.

Most preferably, the peptide comprised in the vaccine of the present invention comprises the amino acid sequence set forth in SEQ ID NO: 2 (TTR34-54) or SEQ ID NO: 3 (TTR39-50).

Relatively short immunogenic peptides, *i.e.,* TTR peptides (less than about 50 amino acids), are usually synthesized using standard chemical peptide synthesis techniques, like solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence. Techniques for solid phase synthesis are known to those skilled in the art. Alternatively, the TTR peptides can be synthesized using recombinant nucleic acid methodology. Generally, this involves creating a nucleic acid sequence that encodes the peptide, placing the nucleic acid in an expression cassette under the control of a particular promoter, expressing the peptide in a host, isolating the expressed peptide or polypeptide and, if required, renaturing the peptide. Techniques sufficient to guide one of skill through such procedures are found in the literature. Once expressed, recombinant peptides can be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like.

In Examples 1 and 2, the superior performance of a cyclic peptide over the corresponding linear peptide in ELISA assays was shown. In particular, the binding affinity of the TTR antibody to the cyclized TTR peptide was about 10- to 100-fold higher than to the corresponding linear peptide. Thus, in one embodiment, the peptide comprised in the vaccine of the present invention forms a cyclic compound. In other words, in one embodiment, the present invention relates to a vaccine comprising a cyclic compound comprising a peptide containing an epitope of a TTR protein, the epitope preferably being accessible to binding by an antibody only in the misfolded and/or aggregated form of the protein, as in the case of a neoepitope, and/or the epitope being at least not present in the physiologically active form of the protein, *e.g.* in the case of an epitope accessible in the monomer of the TTR protein, which is hidden in the physiologically active tetramer and is no longer accessible to antibody binding. In particular, in one embodiment, the vaccine of the present invention comprises the TTR peptide as defined above in a form able to cyclize. In a preferred embodiment, the TTR peptide comprises a linker covalently coupled to the peptide N-terminus residue and the C-terminus residue to form a cyclic compound.

As described in Example 3 and shown in Fig. 3 and 4, both immunization with the cyclic peptides TTR34-54cyc and TTR39-50cyc and surprisingly even more pronounced with the respective linear peptides TTR34-54 and TTR39-50 resulted in a selective immune response in that antibodies specific for ATTR have been generated upon immunization with said peptides as measured by the serum reactivity and amyloid selectively.

Thus, the present invention also relates to the use of the TTR peptide form as immunogen, *i.e.*, immunogenic compound. The vaccine of the present invention can comprise one immunogenic compound, *i.e.*, one kind of TTR peptide, or more than one kind of TTR peptide, for example two, three or four different kinds of TTR peptides.

The linear TTR peptides used in the Examples consist of the amino acid sequence RKAADDTWEPFASGKTSESGE (TTR34-54; SEQ ID NO: 2) with a total of 21 amino acids of the amyloidogenic protein TTR, including the five amino acid epitope WEPFA, and of the amino acid sequence DTWEPFASGKTS (TTR394-50; SEQ ID NO: 3) with a total of 12 amino acids of the amyloidogenic protein TTR, including the five amino acid epitope WEPFA. Thus, in a preferred embodiment, the linear peptide consists of a total of 5 to 30, preferably of 10 to 30, more preferably of 10 to 25 and even more preferably of 20 ± one, two, three or four amino acids, or of 10 ± one, two, three or four amino acids, but most preferably of 20 ± one, two, three or four amino acids.

The cyclic TTR peptides used in the Examples consist of the amino acid sequence GCGGGRKAADDTWEPFASGKTSESGEGGGCG (TTR34-54cyc; SEQ ID NO: 4) with a total of 31 amino acids and comprises 21 amino acids of the amyloidogenic protein TTR, including the five amino acid epitope WEPFA, and linker sequences of 10 amino acids, five amino acids each the N- and C-termini of the 21 amino acid stretch from TTR, and of the amino acid sequence GCGGGDTWEPFASGKTSGGGCG (TTR394-50cyc; SEQ ID NO: 5) with a total of 22 amino acids and comprises 12 amino acids of the amyloidogenic protein TTR, including the five amino acid epitope WEPFA, and linker sequences of 10 amino acids, five amino acids each the N- and C-termini of the 12 amino acid stretch from TTR. Thus, in a preferred embodiment, the cyclic compound consists of a total of 15 to 40, preferably of 20 to 40, more preferably 20 to 35 and even more preferably of 30 ± one, two, three or four amino acids, or of 20 ± one, two, three or four amino acids, but most preferably of 30 ± one, two, three or four amino acids or, in case non-amino acid residues are incorporated, for example as a linker, is configured such that its structure resembles a corresponding peptide. In this embodiment, the amino acid sequence derived from the TTR protein present in the cyclic compound may consist of 10 to 40, preferably of 10 to 25 and more preferably of 20 ± one, two, three or four amino acids or of 10 ± one, two, three or four amino acids, but most preferably of 20 ± one, two, three or four amino acids, and optionally supplemented with a linker,

preferably 5 to 20 amino acids in length, more preferably 5 to 15 and most preferably of 10 ± one, two, three or four amino acids, either distributed on both ends, N- and C-terminus or only at one terminus. It is also conceivable that linker sequences or "filling" sequences are located within amino acid sequence derived from the amyloidogenic protein, *e.g.*, if the epitope of the target binding molecule is a conformational epitope or discontinuous epitope.

Thus, the cyclic compound present in the vaccine of the present invention can either comprise or consist of the TTR peptide which consists of the TTR neoepitope, or which comprises the epitope, meaning that additional amino acids or other chemical entities used for example for cyclization of the peptide or protein fragment as described further below can be present in the protein fragment or peptide which forms the cyclic compound.

The additional amino acids can be amino acids which are naturally located adjacent to the epitope sequence, *i.e.*, amino acids that are flanking the epitope sequence, and which are present in the TTR protein sequence the peptide is derived from, *i.e.*, the TTR peptide which forms the cyclic compound comprises the TTR epitope and further amino acids that are adjacent to the epitope and that are flanking the epitope, respectively. The number of those adjacent/flanking amino acids can vary and can be, for example, between 1, 2, or 3 amino acid and 50 amino acids, preferably between 1, 2, or 3 amino acids and 40 amino acids, more preferably between 1, 2, or 3 and 30 amino acids, more preferably between 1, 2, or 3 and 20 amino acids, more preferably between 10 and 20 amino acids, wherein the amino acids are either distributed equally N-terminal and C-terminal to the epitope sequence or unequally, with for example 7 additional amino acids N-terminal and 9 amino acids C-terminal to the epitope.

In addition, or alternatively, the TTR peptide comprises in one embodiment a linker, *i.e.*, the protein fragment or peptide can either comprise the epitope without any adjacent amino acids, and a linker, or can comprise the epitope and the adjacent amino acids as defined above, and a linker. In a preferred embodiment, the TTR peptide which forms the cyclic compound comprised in the vaccine of the present invention comprises the neoepitope as well as amino acids adjacent to the epitope, and a linker. Preferably, the linker is covalently coupled directly or indirectly to the N-terminus residue of the TTR peptide and to the C-terminal residue of the TTR peptide.

In an embodiment, the linker amino acids are selected from non-immunogenic or poorly immunogenic amino acid residues such as G, or A, for example the linker can be GG, GGG, GAG, G(PEG)G, PEG-PEG (also referred to as PEG2)-GG and the like. One or more functionalizable moieties *e.g.*, amino acids with a functional group may be included for example for coupling the compound to an immunogenicity enhancing agent like a carrier such as BSA.

Methods for cyclization of peptides are generally known in the art. For example, cyclization can be performed by chemical crosslinking using *inter alia* chemical scaffolds. Crosslinking requires functional groups and just few protein chemical targets account for the vast majority of crosslinking techniques, *e.g.*, primary amines ($-NH_2$), wherein this group exists at the N-terminus of each polypeptide chain and in the side chain of lysine residues; carboxyls (–COOH), wherein this group exists at the C-terminus of each polypeptide chain and in the side chains of aspartic acid and glutamic acid; and sulfhydryls (–SH), wherein this group exists in the side chain of cysteine.

Scaffold-based cyclization is one of the most frequently used methods because it can be applied to chemically or biologically synthesized peptides. In general, scaffold compounds such as organohalides (most frequently organobromides) selectively react with the sulfhydryl group of cysteine. Non-sulfhydryl groups, such as the primary amine of lysine or N-terminal amino group in a peptide, can also be used for cyclization for example with N-hydroxysuccinimide (NHS)-containing chemicals. Especially designed unnatural amino acids can also be used for cyclization in peptides via a bio-orthogonal reaction. For example, if an azide-containing amino acid such as azidohomoalanine or azidophenylalanine exists in a peptide, a copper-mediated click reaction with an alkyne-bearing scaffold can lead to cyclization.

Furthermore, cysteines can be joined together between their side chains via disulfide bonds (–S–S–) or amide cyclization can be performed without any scaffold (head-to-tail, or backbone cyclization).

For example, a peptide with "C" residues at its N- and C- termini, *e.g.*, the cyclic TTR compound used in Examples 1 and 2, GCGGGRKAADDTWEPFASGKTSESGEGGGCG (SEQ ID NO: 4) and GCGGGDTWEPFASGKTSGGGCG (SEQ ID NO: 5), can be reacted by S-S-cyclization to produce a cyclic peptide. The cyclic compound can be synthesized as a linear molecule with the linker covalently attached at or near the N-terminus or C-terminus of the

peptide comprising the TTR peptide, or related epitopes mentioned herein prior to cyclization. Alternatively, part of the linker is covalently attached at or near the N-terminus and part is covalently attached at or near the C-terminus prior to cyclization. In either case, the linear compound is cyclized for example by S-S bond cyclization. Accordingly, the compounds may be cyclized by covalently bonding 1) at or near the N-terminus and the C-terminus of the peptide + linker to form a peptide bond (*e.g.,* cyclizing the backbone), 2) at or near the N-terminus or the C-terminus with a side chain in the peptide + linker, or 3) two side chains in the peptide + linker. In this context, "near" is defined as being within 1, 2, or 3 amino acid residues of the N- or C-terminus. Preferably, the linker is coupled to the N-terminus or C-terminus.

As mentioned above, peptides may be cyclized by oxidation of thiol- or mercaptan-containing residues at or near the N-terminus or C-terminus, or internal to the peptide, including for example cysteine and homocysteine. For example, two cysteine residues flanking the peptide may be oxidized to form a disulphide bond. Oxidative reagents that may employed include, for example, oxygen (air), dimethyl sulphoxide, oxidized glutathione, cystine, copper (II) chloride, potassium ferricyanide, thallium(III) trifluro acetate, or other oxidative reagents such as may be known to those of skill in the art and used with such methods as are known to those of skill in the art. Crosslinking agents are also known in the art and can be chosen for example based on the functional groups to be used for crosslinking, see for example the Crosslinker Selection Tool provided by Thermo Fisher Scientific.

Accordingly, in one embodiment, the linker comprises a functionalizable moiety, *e.g.*, an amino acid with one of the above-mentioned functional groups such as lysine, aspartic acid, glutamic acid, or cysteine, a non-naturally occurring amino acid such as azidohomoalanine or azidophenylalanine, or equivalently functioning molecules such as polyethylene glycol (PEG).

In case the functionalizable moiety is a naturally occurring amino acid, such as lysine, aspartic acid, glutamic acid, serine, threonine, or cysteine, the functionalizable moiety does not necessarily have to be in the linker but can also be present in the epitope or within the adjacent amino acids present in the protein fragment or peptide forming the cyclic peptide. Thus, cyclization of the TTR peptide can also be performed without a linker. Accordingly, in one embodiment, the TTR peptides forms the cyclic compound present in the vaccine of the present invention without a linker. The linkage may occur via the side chain of one or more amino acids, such as the sulfhydryl moiety of a cysteine residue, the carboxylic acid moiety of an

aspartic acid or glutamic acid residue, the hydroxyl of a serine or threonine residue, or the amine of a lysine or arginine residue.

In a preferred embodiment, the at least one functionalizable moiety is present in the linker, *i.e.*, the linker comprises one or more functionalizable moieties. The linker can comprise or consist of any amino acids including non-natural amino acids, but preferably comprises at least any one of the functionalizable moieties mentioned above, *i.e.*, lysine, aspartic acid, glutamic acid, or cysteine, non-naturally occurring amino acids such as azidohomoalanine or azidophenylalanine, or equivalently functioning molecules such as polyethylene glycol (PEG). In one embodiment, the linker comprises one or more PEG molecules as functionalizable moiety. In a preferred embodiment, the linker comprises cysteine as functionalizable moiety.

Accordingly, in a preferred embodiment, the linker of any length and sequence can be described with the following sequence X-nX-1FX1-Xn, wherein F is any functionalizable moiety, preferably C (cysteine), and X any amino acid including non-natural amino acids. In a further preferred embodiment, the linker amino acids are selected from alanine (A), or glycine (G), or serine (S), or from alanine (A) and glycine (G), or from glycine (G) and serine (S), but preferably glycine (G).

Even more preferred, the linker amino acids are selected from alanine (A), or glycine (G), or serine (S), or from alanine (A) and glycine (G), or from glycine (G) and serine (S), preferably glycine (G) and the functionalizable moiety is cysteine (C). Accordingly, preferably, the cyclization is performed with scaffold compounds such as organohalides, preferably organobromides, that selectively react with the sulfhydryl group of cysteine, or via a disulfide bridge. Most preferably, cyclization is performed via a disulfide bridge.

In a preferred embodiment, the linker comprises 1 to 40 amino acids, preferably 1 to 35 amino acids, more preferably 1 to 30 amino acids, more preferably 1 to 25 amino acids, more preferably 1 to 20 amino acids, more preferably 1 to 10 amino acids, more preferably 1 to 9 amino acids, and most preferably 1 to 8 amino, in particular 1, 2, 3, 4, 5, 6, 7, or 8 amino acids and/or equivalent functioning molecules, and/or a combination thereof, wherein, when the linker comprises only amino acids, there is preferably within the amino acids at least one amino acid having any of the above-mentioned functional groups, preferably cysteine. The other

amino acids comprised in the linker can be chosen from any known amino acids including non-natural amino acids, but are preferably alanine (A) and/or glycine (G), preferably glycine (G).

As mentioned above, the length of the linker can vary and can be for example 9 amino acids, for example GGGGCGGGG (SEQ ID NO: 27), or 8 amino acids, for example GGGCGGGG (SEQ ID NO: 28), GGCGGGGG (SEQ ID NO: 29) or GCGGGGGG (SEQ ID NO: 30), or 7 amino acids, for example GGGGCGG (SEQ ID NO: 31), GGGCGGG (SEQ ID NO: 32), GGCGGGG (SEQ ID NO: 33) or GCGGGGG (SEQ ID NO: 34), 6 amino acids, for example GGGCGG (SEQ ID NO: 35), GGCGGG (SEQ ID NO: 36) or GCGGGG (SEQ ID NO: 37), 5 amino acids, for example GCGGG (SEQ ID NO: 25) or GGGCG (SEQ ID NO: 26), 4 amino acids such as GCGG (SEQ ID NO: 38) or GGCG (SEQ ID NO: 39) or 3 amino acids such as GCG. Most preferably, the linker in the cyclic compound comprises or consists of GCGGG (SEQ ID NO: 25) or GGGCG (SEQ ID NO: 26).

As mentioned above, the cyclic compound comprises a peptide comprising a TTR epitope, and most preferably the epitope comprising the amino acid sequence WEPFA (SEQ ID NO: 1) as well as adjacent amino acids and a linker at the peptide N-terminus and C-terminus, wherein the linker can in principle comprise any of the above-described linker sequences, and preferably comprises the amino acid sequence GCGGG (SEQ ID NO: 25) or GGGCG (SEQ ID NO: 26). Thus, a preferred embodiment, the cyclic compound comprises or consists of the amino acid sequence H-GCGGGRKAADDTWEPFASGKTSESGEGGGCG-OH (TTR34-54cyc; SEQ ID NO: 4) or H-GCGGGDTWEPFASGKTSGGGCG-OH (TTR39-50cyc; SEQ ID NO: 5), which have been shown in Examples 1 and 2 as suitable target antigens.

In some cases, peptides have a limited immunogenicity. Techniques for conferring immunogenicity on a peptide are well known in the art and include, for example, conjugation to an immunogenicity enhancing agent, like carriers or T helper cell epitopes, or administration in the presence of an adjuvant.

In one embodiment, the TTR peptide as comprised in the vaccine of the present invention is formulated with an immunogenicity enhancing agent, *i.e.*, with an agent helping to elicit an immune response against the peptide.

In one embodiment, the TTR peptide as comprised in the vaccine of the present invention further comprises a carrier, like BSA as used in Example 2. Proteins, such as BSA and/or other

agents may be coupled to the TTR peptide for example to aid in immunogenicity. Suitable carrier for this purpose are known in the art and include for example, but are not limited to maltose binding protein "MBP", bovine serum albumin (BSA), KLH (keyhole limpet hemocyanin), ovalbumin, flagellin, serum albumins, immunoglobulin molecules, thyroglobulin, ovalbumin, polymers of D-and/or L- amino acids, tetanus toxoid (TT), diphtheria toxoid (DT), a genetically modified cross-reacting material (CRM) of diphtheria toxin, CRM197, meningococcal outer membrane protein complex (OMPC) and *H influenzae* protein D (HiD), and *Pseudomonas aeruginosa* exotoxin A. Various methods for chemical crosslinking of peptides to carrier proteins are known in the art and typically involve reactive sulfuydryl and/or amino groups. Many of these systems are commercially available *(e.g.*, Imject™ from ThermoFisher Scientific). Most commonly chemical crosslinking is done using a sulfuydryl group in the antigen, which can be introduced by addition of a cysteine residue to the antigenic peptide, *e.g.*, C-(GA)10, or via a primary amino group *(e.g.*, Imject™ Maleimide and Imject™ EDC products from ThermoFisher Scientific). Furthermore, methods for conjugating peptides to an immunogenicity enhancing agent such as KLH or a carrier such as BSA are described in Lateef *et al.*, Journal of Biomolecular Techniques 18 (2007), 173–176, herein incorporated by reference.

In one embodiment, the carrier coupled to the TTR peptide is BSA or KLH, *i.e.*, the TTR peptide comprises a carrier and an immunogenicity enhancing agent, respectively, such as BSA or KHL. In a preferred embodiment, the carrier coupled to the TTR peptide is BSA, *i.e.*, the TTR peptide comprises a carrier and an immunogenicity enhancing agent, respectively, such as BSA. The immunogenicity enhancing agent/carrier can be coupled to the peptide either directly, such as through an amide bound or disulfide bond, or indirectly through a chemical linker. In particular, BSA is covalently coupled using a free amine reaction connecting free amines on the peptide with free amines on the BSA protein.

It is also known that the use of KLH may result in the generation of a large amount of antibodies directed against KLH, which is an unwanted side effect and thus, should be avoided. Accordingly, in one embodiment, the vaccine does not comprise KLH as immunogenicity enhancing agent.

In one embodiment, the immunogen comprising the TTR peptide and the TTR peptide, respectively, comprises a T helper cell epitope. More particularly, the TTR peptide can be

linked to a heterologous T helper cell epitope peptide to form a peptide immunogen construct. Optionally, linking can be performed through a heterologous spacer. The term "heterologous", as used herein, refers to an amino acid sequence that is derived from an amino acid sequence that is not part of, or homologous to the wild-type sequence of TTR. The heterologous spacer can be any molecule or chemical structure capable of linking two amino acids and/or peptides together, which can include a chemical compound, a naturally occurring amino acid, a non-naturally occurring amino acid, or any combination thereof. The heterologous T helper cell epitope can be any T helper cell epitope that is capable of enhancing the immune response to the TTR epitope. The T helper cell epitope can also have promiscuous binding motifs to MHC class II molecules of multiple species or may comprises multiple promiscuous MHC class II binding motifs to allow maximal activation of T helper cells leading to initiation and regulation of immune responses. The T helper cell epitope is preferably immunosilent on its own, i.e. little, if any, of the antibodies generated by the TTR peptide immunogen constructs will be directed towards the T helper cell epitope, thus allowing a very focused immune response directed to the targeted TTR epitope peptide. T helper cell epitopes can include amino acid sequences derived from foreign pathogens. Several universal T helper cell epitopes are known in the art which can be used herein, for example universal T helper cell epitope from tetanus and diphtheria toxins, like P30 (Deithelm-Okita et al., The Journal of Infectious Diseases 181 (2000), 1001–1009; Swartz et al., npj Vaccines 6 (2021),12.

Accordingly, in one embodiment, the peptide is formulated with an immunogenicity enhancing agent, preferably linked to a carrier molecule, like a carrier protein, such as BSA, or to a T helper cell epitope, to form a conjugate which helps elicit an immune response against the peptide, preferably whereby the induced antibodies specifically bind to and clear amyloidogenic TTR, *i.e.,* TTR aggregates in a subject thereby inhibiting the formation of TTR deposits and thereby effecting treatment of prophylaxis of the disease.

Alternatively, the immunogen may be a multi antigenic peptide (MAP) comprising the TTR peptide. Multiple antigen-presenting peptide vaccine systems have been developed to avoid the adverse effects associated with conventional vaccines, *i.e.,* live-attenuated, killed or inactivated pathogens), carrier proteins and cytotoxic adjuvants. Two main approaches have been used to develop multiple antigen presenting peptide vaccine systems: (1) the addition of functional components, *e.g.,* T-cell epitopes, cell-penetrating peptides, and lipophilic moieties; and (2) synthetic approaches using size-defined nanomaterials, *e.g.,* self-assembling peptides, non-

peptidic dendrimers, and gold nanoparticles, as antigen-displaying platforms. Use of a multiple antigenic peptide (MAP) system can improve the sometimes poor immunogenicity of subunit peptide vaccines. In a MAP system, multiple copies of antigenic peptides are simultaneously bound to the a- and £-amino groups of a non-immunogenic Lys-based dendritic scaffold, helping to confer stability from degradation, thus enhancing molecular recognition by immune cells, and induction of stronger immune responses compared with small antigenic peptides alone. In some compositions, the MAP comprises one or more of a Lys-based dendritic scaffold, helper T-cell epitopes, immune stimulating lipophilic moieties, cell penetrating peptides, radical induced polymerization, self-assembling nanoparticles as antigen-presenting platforms and gold nanoparticles. Thus, in one embodiment, the TTR peptide comprised in the vaccine of the present invention is prepared as MAP.

The present invention does not include a MAP, in which the sequence GGEHAEVVFTAGGGK is synthesized in eight copies on a MAP dendritic core ([Fluorenylmethyloxycarbonyl (Fmoc) Fmoc-Lys (Fmoc)] 4-Lys2-Lys-bAla) attached to a Wang resin. This MAP is described in Higaki *et al.*, Amyloid, 23:2 (2016), 86-97 and illustrated in Bugyei-Twum 2012 (Inhibition of Transthyretin Fibrillogenesis Using a Conformation Specific Antibody by Antoinette Bugyei-Twum, a thesis submitted in conformity with the requirements for the degree of Master of Science, Graduate Department of Biochemistry, University of Toronto).

In one embodiment, the vaccine of the present invention comprises, next to the TTR peptide, an adjuvant. Most vaccines are injected with an adjuvant to stimulate an immune response. The incorporation of adjuvants into vaccine formulations is aimed at enhancing, accelerating and prolonging the specific immune response towards the desired response to vaccine antigens. Advantages of adjuvants include the enhancement of the immunogenicity of antigens, modification of the nature of the immune response, the reduction of the antigen amount needed for a successful immunization, the reduction of the frequency of booster immunizations needed and an improved immune response in elderly and immunocompromised patients. The adjuvant usably to exercise the invention includes in general any adjuvant known in the art that boosts the immune response to the immunogenic composition, *i.e.*, the vaccine, and/or results in a longer-lasting immunity without resulting in an immune response by themselves. Preferred adjuvants allow the reduction of the immunogenic composition to be applied for a sufficient immune response. However, the nature of adjuvants can vary extensively. For example,

conformationally designed epitopes may require adjuvants that do not denature or emulsify the antigens. Thus, in one embodiment, the vaccine of the present invention comprises an adjuvant, preferably such an adjuvant that does not have an influence of the structure of the peptide, for example the cyclized structure.

Suitable adjuvants can be selected and evaluated by a person skilled in the art, for example based on the "Guideline on adjuvants in vaccine for human use" of the European Medicines Agency of January 20, 2005, last update February 16, 2023 (EMEA/CHMP/VEG/134716/2004). The adjuvant may be administered with an immunogen as a single composition. Alternatively, an adjuvant may be administered before, concurrent and/or after administration of the immunogen. Exemplarily adjuvants are listed above and in the following: There are different kinds of adjuvant groups that can be used, *i.e.*, (i) delivery systems, such as mineral salts, *e.g.,* aluminum salts (aluminum hydroxide, aluminum sulfate and aluminum phosphate), emulsions, *e.g.,* Freund's adjuvants, MF59, or AS03, and microparticles, *e.g.,* virus-like particles, virosomes, PLA/PLGA, (ii) immune potentiators, such as TLR1/2 agonists, *e.g.*, L-pampo, MALP-2, Pam2CSK4 and Pam3CSK4, TLR3 agonists, *e.g.,* Poly(I:C) (polyinosinic:polycytidylic acid) Poly-ICLC, TLR4 agonists, *e.g.*, Monophosphoryl lipid A (MPL), TLR5 agonists, *e.g.,* Flagellin, TLR7/8 agonists, *e.g.,* Imiquimod (R837; 1-(2-methylpropyl)-1H-imidazo [4,5-c]quinolin-4-amine) and resiquimod (R848, 4-amino-2-(etoximetil)-a,a-dimethyl-1H-imidazo [4,5-c]quinoline-1-ethanol), and TLR9 agonists, *e.g.,* CpG ODNs, (iii) combined adjuvants, *e.g.*, AS01 and AS02, AS04, and (iv) mucosal adjuvants, *e.g.*, Cholera toxin (CT), Heat-labile enterotoxin (LTK3 and LTR72), Chitosan; see for review Facciolà *et al.,* Vaccines 10 (2022), 819. Some adjuvants preferably used include Incomplete Freund's adjuvant (IFA) (Montanide ISA-51) or CpG oligodeoxynucleotides (ODNs).

In accordance with the present invention, the immunogen for the vaccine of the present invention can be synthesized chemically or produced using recombinant DNA techniques, wherein the TTR peptide disclosed herein may be coupled or not to an immunogenic carrier. Corresponding means and methods are well-known in the art; see, *e.g.*, Hou *et al.*, Trans. Tianjin Univ. 23 (2017), 401–41; https://doi.org/10.1007/s12209-017-0068-8; Molecular Biotechnology: Principles and Applications of Recombinant DNA, 6th Edition, Eds. Glick and Patten; ISBN: 978-1-683-67366-8 February 2022; Textbook on Cloning, Expression and Purification of Recombinant Proteins, 2022 Ed. Kakoli Bose; ISBN: 978-981-16-4986-8.

Accordingly, the present invention also relates to a nucleic acid encoding the immunogen as described herein, preferably in combination with an immunogenicity enhancing agent, like an immunogenic carrier or adjuvant such as one of those described above.

Typically, the nucleic acid of the present invention is in an expressible form, *i.e.*, when subjected to a cell, the immunogen or part thereof is expressed from the nucleic acid. In one embodiment, this can be achieved by using a translatable nucleic acid such as mRNA, for example for use in *in vitro* translation or by incorporating the nucleic acid in an expression vector capable of expressing the nucleic acid of claim, which thus also forms part of the present invention.

In a further embodiment, the present invention relates a host cell comprising a nucleic acid or an expression vector of the present invention as well as to the use of the nucleic acid and expression vector for producing the immunogen in a cell. The cell could be, for example, a bacterial, yeast, or mammalian cell.

In one embodiment, the immunogen may be purified from the cell culture and formulated as a vaccine, optionally including a step of coupling the immunogen, *e.g.*, TTR peptide to immunogenicity enhancing agent, such as immunogenic carrier and/or adjuvants. Thus, a method of recombinantly producing the immunogen by expressing it in a host cell and isolating the immunogen therefrom is a further aspect of the invention. Any suitable host cell, e.g. prokaryotic or eukaryotic, can be used, in particular embodiments the host cell is selected from the group consisting of a bacterial, fungal (including yeast), and mammalian cell.

The present invention also relates to a kit and composition comprising a nucleic acid encoding the immunogen of the present invention, either including or not an immunogenicity enhancing agent, like an immunogenic carrier, an expression vector comprising the nucleic acid, a host cell comprising the nucleic acid or the expression vector, and/or the immunogen of the present invention, and optionally at least one immunogenicity enhancing agent, like an immunogenic carrier, if applicable, and/or adjuvant.

The present invention further relates to a kit comprising the vaccine of the present invention, and which optionally comprises means and/or instructions for administering of the vaccine, for

example a dosing recommendation and/or a syringe for administering the vaccine. In a preferred embodiment, the kit comprises the vaccine of the present invention which is present in a pharmaceutical container, like a prefilled vial or syringe.

Moreover, a method is disclosed for the preparation of a vaccine composition for inducing an immune response in an organism, in particular an animal or human affected by ATTR or a healthy organism being at risk of developing ATTR, and thus in need of such a treatment, for preventing, treating or alleviating the effects of ATTR, the method comprises formulating the TTR peptide according to the invention in a pharmaceutically acceptable form. Pharmaceutical acceptable carriers for the formulation of vaccines are known in the art.

As explained above, the vaccine of the present invention can be used for the prevention of ATTR in a subject via triggering the immune response, *e.g.,* via eliciting anti-TRR antibodies capable of binding to and clearing amyloidogenic TTR, *i.e.,* TTR aggregates. Anti-TTR antibodies have been shown to be useful for the treatment of ATTR, for example of ATTR-CM; see Garcia-Pavia *et al,* 2023, *supra*. The results described in Garcia-Pavia *et al.* further point towards the usefulness of anti-TTR antibodies in the treatment of musculoskeletal disorders since as illustrated in Figure 2 of Garcia *et al.*, TTR deposits are cleared in the shoulder joints during treatment with antibody NI006. Thus, the vaccine of the present invention may also be useful in the treatment of ATTR, in particular of early stages of ATTR in subject. Accordingly, the present invention also relates to the vaccine and the kit, respectively of the present invention for use in the prevention or treatment of ATTR, for example ATTR cardiomyopathy (ATTR-CM), ATTR polyneuropathy (ATTR-PN), in particular of ATTR-CM and of a musculoskeletal disorder or condition in a subject, wherein the latter one is preferably associated with deposition of TTR in the joints and is most preferably selected from the group consisting of osteoarthritis, carpal tunnel syndrome, joint pain, shoulder pain, amyloid arthropathy, lumbar spine stenosis, brachial biceps tendon rupture, trigger finger, and rotator cuff disease, preferably wherein the musculoskeletal disorder or condition is osteoarthritis or amyloid arthropathy, preferably amyloid arthropathy.

The progress of immunization with the vaccine of the present invention can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassay procedures can be used with the immunogen as antigen to assess antibody levels. Following

immunization, antisera can be obtained and, if desired, polyclonal antibodies isolated from the sera; see also Example 3.

ATTR includes two sub-types - wild-type ATTR (ATTRwt) and variant ATTR (ATTRv). In 10 - 15% of individuals over the age of 65 cardiac TTR deposits due to ATTRwt are found. The epitope comprising the amino acid sequence WEPFA (SEQ ID NO: 1), can be found in the TTRwt and thus, in one embodiment, the vaccine of the present invention is particularly suitable for the treatment or prevention of a subject having sporadic, wild-type ATTR, preferably wild type ATTR-CM. The vaccine of the present invention is also particularly suitable for the treatment or prevention of a subject having a negative genetic testing for a TTR mutation.

The present invention further relates to a method of treating or preventing ATTR, for example ATTR-PN, or ATTR-CM, in particular ATTR-CM and the above-mentioned musculoskeletal disorder, preferably such disorders are associated with the accumulation of wild type TTR, in a subject comprising administering the vaccine of the present invention in an effective amount. Administration can be performed by commonly known techniques, *e.g.*, via intravenous, intramuscular, intradermal or subcutaneous routes of administration.

The vaccine of the present invention may be administered to a patient population at risk for developing ATTR, for example an advanced age population, or a known population of "at risk" individuals harboring a known ATTR-promoting mutation. More particularly, subjects amenable to treatment include individuals at risk of the disease but not showing symptoms, as well as patients presently showing symptoms, including treatment naive subjects that have not been previous treated for the disease. Subjects at risk of the disease include those in an aging population, and asymptomatic subjects having a known genetic risk of disease. Such individuals include those having relatives who have experienced this disease, and those whose risk is determined by analysis of genetic or biochemical markers.

In prophylactic applications, the vaccine of the present invention can be administered to a subject susceptible to, or otherwise at risk of ATTR in a regimen (dose, frequency and route of administration) effective to reduce the risk, lessen the severity, or delay the onset of at least one sign or symptom of the disease. In particular, the regimen is effective to inhibit or delay amyloidogenic TTR formation.

In therapeutic applications, the vaccine of the present invention may be administered to a subject suspected of, or a patient already suffering from ATTR in a regimen (dose, frequency and route of administration) effective to ameliorate or at least inhibit further deterioration of at least one sign or symptom of the disease. In particular, the regimen is preferably effective to reduce or at least inhibit further increase of levels amyloidogenic TTR.

A regimen is considered therapeutically or prophylactically effective if an individual treated achieves an outcome more favorable than the mean outcome in a control population of comparable subjects not treated by methods of the invention, or if a more favorable outcome is demonstrated in treated subjects versus control subjects in a controlled clinical trial. Effective doses of vary depending on many different factors, such as means of administration, target site, physiological state of the patient, whether other medications administered, and whether treatment is prophylactic or therapeutic.

Several documents are cited throughout the text of this specification. The contents of all cited references (including literature references, issued patents, published patent applications as cited throughout this application including the background section and manufacturer's specifications, instructions, etc.) are hereby expressly incorporated by reference; however, there is no admission that any document cited is indeed prior art as to the present invention.

A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLES

Example 1: TTR peptides display neoepitope of TTR amyloid

To test the immunogenic potential of TTR peptides, *i.e.*, the ability to elicit protective antibodies, which requires binding of the peptide to the antibody, corresponding binding studies were initially performed.

In particular, the ability of an anti-TTR antibody to bind a TTR peptide has exemplarily been evaluated with ELISA assays using a cyclic peptide comprising the amino acid residues 34 to 54 of wild type TTR (TTR34-54cyc in biotinylated and non-biotinylated form) and a corresponding linear peptide (TTR34-54) in biotinylated form as target antigens and the

antibody NI-301.37F1 as anti-TTR antibody. Furthermore, as antigen control, the TTR peptide TTR40-49 as well as misfolded wild type TTR (mis.WT-TTR) were used.

The cyclic peptide TTR34-54cyc (1.36 mg/mL) has been manufactured by Schafer-N (Copenhagen, Denmark) and stored at -20°C. In particular, the peptide comprising the amino acid sequence H-GCGGGRKAADDTWEPFASGKTSESGEGGGCG-OH (SEQ ID NO: 4) has been synthesized by solid phase peptide synthesis and cyclized via disulfide bridge between two cysteine residues within the poly-glycine stretch. The TTR peptides comprising the amino acid sequences H-RKAADDTWEPFASGKTSESGE-OH (SEQ ID NO: 2, TTR34-54) and H-TWEPFASGKT-OH (SEQ ID NO: 6, TTR40-49, 1.25 mg/mL) have also been manufactured by Schafer-N (Copenhagen, Denmark) and stored at -20°C. The biotinylated peptides TTR34-54cyc_bt and TTR34-54_bt comprise an amino hexanoic acid (Ahx) spacer between their N-terminus and the biotin residue, *i.e.*, TTR34-54cyc_bt (Biotin-(Ahx)GCGGGRKAADDTWEPFASGKTSESGEGGGCG-OH (SEQ ID NO: 4), 680 µg/mL) and TTR34-54_bt (Biotin-(Ahx)RKAADDTWEPFASGKTSESGE-OH (SEQ ID NO: 2). Wild-type TTR protein purified from human plasma was obtained from Bio-Rad Laboratories, Inc. (California, USA; 7600-0604) and submitted to a custom purification through protein A/G chromatography followed by a lectin column to eliminate residual immunoglobulins. Plasma-purified WT-TTR was provided as a solution at a concentration of 1 mg/ml in PBS buffer. Misfolded WT-TTR aggregates (mis.WT-TTR) were prepared *in vitro* by diluting WT-TTR stock solutions to a concentration of 200 µg/ml in aggregation buffer (50 mM acetate-HCl, 100 mM KCl, 1 mM EDTA, pH 3.0) followed by incubation for 4 hours at 37°C with shaking at 1000 rpm. mis.WT-TTR was aliquoted and stored until use at -20°C. The quality of mis.WT-TTR was confirmed by ELISA and Biolayer interferometry (BLI).

Three ELISA assays have been performed, wherein in the first ELISA assay (ELISA-1), antibody binding to the peptide TTR34-54cyc and mis-WT-TTR has been analyzed, in the second ELISA assay (ELISA-2), antibody binding to the peptides TTR34-54_bt and TTR34-54cyc_bt as well as to a BSA control has been analyzed, and in the third ELISA assay (ELISA-3), antibody binding to the peptides TTR34-54cyc and TTR40-49 has been analyzed.

In particular, 96-well microplates were coated for 1 hour at 37°C with TTR34-54cyc and mis-WT-TTR (ELISA-1), with TTR34-54_bt, TTR34-54cyc_bt and BSA (ELISA-2), and with TTR34-54cyc and TTR40-49 (ELISA-3), respectively, wherein each target antigen has been

diluted to a concentration of 10 µg/ml in PBS buffer, pH 7.4. Non-specific binding sites were blocked for 1 hour at room temperature (RT) with a blocking buffer containing 2% (w/v) bovine serum albumin (BSA) and 0.1 % polysorbate 20 in 1 x PBS buffer, pH 7.4. NI-301.37F1 antibody (Neurimmune AG, Zurich, Switzerland; NI-301.37F1) was diluted in duplicates to the indicated concentrations (dilution series from 400 nM to 4 pM and 0) in the blocking buffer and incubated overnight at 4°C. Binding was determined using an anti-human IgG antibody conjugated with horseradish peroxidase (HRP), followed by measurement of HRP activity in a standard colorimetric assay (ThermoFisher Scientific Inc., Waltham, Massachusetts, USA). Data were analyzed with the Prism software from GraphPad. EC_{50} values were estimated using non-linear regression of individual data points using log(agonist) versus response model with variable slope. Data fitting was performed with the least square regression method.

The ELISA results showed that antibody NI-301.37F1 binds to TTR peptides. The ELISA-1 assay showed that NI-301.37F1 binding to the cyclic TTR34-54cyc peptide is much stronger, *i.e.*, about 10-fold stronger, than binding to mis.WT-TTR. In particular, in ELISA-1, NI-301.37F1 binding EC_{50} for the cyclic TTR34-54cyc peptide was 0.022 nM and NI-301.37F1 binding EC_{50} for the mis.WT-TTR was 0.19 nM; see Fig. 1A. It was further shown in the ELISA-2 assay that both linear as well as cyclic TTR peptides were bound by NI-301.37F1, but that cyclization promotes NI-301.37F1 binding by more than 100-fold. In particular, in the ELISA-2 assay, NI-301.37F1 binding EC_{50} for the cyclic TTR34-54cyc_bt peptide was 0.11 nM and NI-301.37F1 binding EC_{50} for the linear TTR34-54_bt was 19.5 nM; see Fig. 1B. No binding of NI-301.37F1 to TTR40-49 was observed in the control ELISA assay, *i.e.*, in the ELISA-3; assay see Fig. 1C.

Example 2: BSA-coupled TTR peptides retain neoepitope of TTR amyloid specific antibody

In typical peptide vaccination protocols, the peptide comprising the epitope of interest is conjugated to a carrier protein, for example to boost immune responses by increasing the half-life of the epitope by decreasing renal clearance and susceptibility to proteolytic degradation. Accordingly, further ELISA assays have been performed evaluating the ability of an anti-TTR antibody, here exemplarily tested with antibody NI-301.37F1, to bind to BSA-coupled TTR peptides.

In particular, the ability of an anti-TTR antibody to bind a BSA-coupled TTR peptide has exemplarily been evaluated with ELISA assays using cyclic peptides comprising the amino acid

residues 34 to 54 of wild type TTR coupled to BSA (TTR34-54cyc_BSA) and comprising the amino acid residues 39 to 50 of wild type TTR coupled to BSA (TTR39-50cyc_BSA) as well as corresponding linear peptides coupled to BSA (TTR34-54_BSA and TTR39-50_BSA) as target antigens and the antibody NI-301.37F1 as anti-TTR antibody. Furthermore, as antigen controls, scrambled cyclic and linear peptides coupled to BSA (TTR34-54SCRcyc_BSA, TTR39-50SCRcyc_BSA, TTR34-54SCR_BSA, and TTR39-50SCR_BSA) have been used.

The cyclic peptides coupled to BSA, *i.e.*, TTR34-54cyc_BSA, TTR39-50cyc_BSA, TTR34-54SCRcyc_BSA, and TTR39-50SCRcyc_BSA, have been manufactured by Schafer-N (Copenhagen, Denmark) and stored at -20°C. In particular, the peptides comprising the amino acid sequence H-GCGGGRKAADDTWEPFASGKTSESGEGGGCG-OH (SEQ ID NO: 4, TTR34-54cyc_BSA), H-GCGGGDTWEPFASGKTSGGGCG-OH (SEQ ID NO: 5, TTR39-50cyc_BSA), H-GCGGGERDDPFKTAWATASGKESESGGGGCG-OH (SEQ ID NO: 9, TTR34-54SCRcyc_BSA), and H-GCGGGEWSDTPTFKGSAGGGCG-OH (SEQ ID NO: 10, TTR39-50SCRcyc_BSA) have been synthesized by solid phase peptide synthesis and cyclized via disulfide bridge between two cysteine residues within the poly-glycine stretch. Furthermore, BSA has been coupled to the cyclic peptides using a bivalent free-amine coupling reagent. The TTR peptides comprising the amino acid sequences H-RKAADDTWEPFASGKTSESGE-OH (SEQ ID NO: 2, TTR34-54_BSA), H-DTWEPFASGKTS-OH (SEQ ID NO: 3, TTR39-50_BSA), H-ERDDPFKTAWATASGKESESG-OH (SEQ ID NO: 7, TTR34-54SCR_BSA), and H-EWSDTPTFKGSA-OH (SEQ ID NO: 8, TTR39-50SCR_BSA) have also been manufactured by Schafer-N (Copenhagen, Denmark) and stored at -20°C.

Misfolded WT-TTR aggregates have been manufactured as described in Example 1.

Two ELISA assays have been performed, wherein in the first ELISA assay (ELISA-1), antibody binding to the peptides TTR34-54cyc_BSA, TTR34-54_BSA, TTR34-54SCRcyc_BSA, and TTR34-54SCR_BSA has been analyzed, and in the second ELISA assay (ELISA-2), antibody binding to the peptides TTR39-50cyc_BSA, TTR39-50_BSA, TTR39-50SCRcyc_BSA, and TTR39-50SCR_BSA has been analyzed. The ELISA assays have been performed as described in Example 1.

The ELISA results showed that antibody NI-301.37F1 binds to BSA-coupled TTR peptides. The ELISA-1 assay showed a high binding affinity of antibody NI-301.37F1 to the cyclic BSA coupled TTR34-54cyc_BSA peptide (EC_{50} value: 3.8 nM) and showed also binding of said

antibody to the corresponding BSA-coupled linear peptide (TTR34-54_BSA) (EC_{50} value: >400 nM). The cyclization promotes NI-301.37F1 binding by more than 100-fold. No binding to the antigen controls, *i.e.,* to the scrambled peptides, was observed; see Fig. 2A. The ELISA-2 assay shows binding to the cyclic BSA coupled TTR39-50cyc_BSA peptide (EC_{50} value: >400 nM), but binding to the corresponding linear peptide could hardly be detected. No binding to the antigen controls, *i.e.,* to the scrambled peptides, was observed. Thus, TTR39-50 is about the minimal peptide length for NI-301.37F1binding; see Fig. 2B.

Example 3: Immunogenic potential of TTR peptides in mammal

Verification of the immunogenic potential of TTR peptides, *i.e.*, that immunization with the TTR peptides will trigger immune responses presenting a high selectivity for amyloidogenic TTR, has been performed in *in vivo* mouse studies. In particular, groups of laboratory animals have been immunized with the target peptides coupled to the carrier protein BSA (coupling to BSA has been performed using amine-reactive cross-linker), with the peptides in a conformation resembling the one present in TTR amyloid fibrils. After repeated injections of the immunogenic peptides, the immune response was monitored by serum titration to characterize its capacity to bind specifically the target protein in amyloid conformation.

Immunization

5 groups of 6 BalbC mice each (total 30 mice) have been injected with BSA-coupled TTR peptides in linear or cyclic conformations. Peptides of 12 and 21 amino-acid length in cyclic and linear conformations have been used to trigger a specific immune response against ATTR (groups 1 to 4). An unrelated peptide, herein referred to as peptide PR906, was used as control to characterize response selectivity.

Peptides were injected s.c. in mice in 3 successive injections each about two weeks apart. Antigens were mixed with RIBI adjuvants for the first and second injection. Pre harvested injection was administered without adjuvants. Blood sampling occured before the first injection (day 0) and after 38 days, *i.e.,* after the third injection. Antigen specific titers against the peptide-antigen and ATTR were determined by ELISA.

List of antigens and immunization groups

- Group 1 TTR34-54_BSA
- Group 2 TTR34-54cyc_BSA
- Group 3 TTR39-50_BSA

- Group 4 TTR39-50cyc_BSA
- Group 5 PR906

Characterization of immune response

Immune response monitoring (serum titer monitoring) and immune response characterization were performed by ELISA against WT-TTR and mis.WT-TTR, also referred to as ATTR, as follows: ELISA plates were coated for 1 hour at 37°C with WT-TTR and mis.WTT-TTR diluted at 10 ug/mL in PBS. Plates were then blocked with a BSA-free blocking buffer for 1 hour at room temperature. Serum samples were diluted as dilution series from 1:100 to 1:5'904'900 in PBS, transferred to the respective ELISA plates and incubated for 1 hour at room temperature. After washing, immunogen-binding antibodies were detected with an HRP-coupled anti-mouse IgG secondary antibody at 1:20'000 dilution.

Immune response monitoring was performed at day 0 and day 38, and afterwards the mice were sacrificed, and final bleed collected. Each serum was tested individually to determine the immune response rate per immunogen.

As shown in Fig. 3, all administered TTR peptides elicit the formation of antibodies against ATTR and TTR, wherein the highest OD_{450} values were detected in samples of sera collected from mice after a 38-day incubation phase. Furthermore, it is evident that the sera of all mice contain significantly higher levels of ATTR-specific antibodies than of TTR-specific antibodies. Accordingly, after immunization of BalbC mice with the respective TTR peptides, serum antibody titers increased over time (0 days compared to 38 days) and the serum reactivity was higher towards mis.WT-TTR/ATTR than towards WT-TTR. In particular, serum obtained from mice after immunization with the peptide/immunogen TTR39-50 showed a serum reactivity (EC_{50}) towards TTR of 1038 and towards ATTR of 15271 and thus, an amyloid selectivity ($EC_{50(ATTR/TTR)}$ quotient) of 14.7; see Fig. 3A and Table 1. Serum obtained from mice after immunization with the peptide/immunogen TTR39-50cyc showed a serum reactivity (EC_{50}) towards TTR of 1813 and towards ATTR of 24343 and thus, an amyloid selectivity ($EC_{50(ATTR/TTR)}$ quotient) of 15.4; see Fig. 3B and Table 1. Serum obtained from mice after immunization with the peptide/immunogen TTR34-54 showed a serum reactivity (EC_{50}) towards TTR of 3400 and towards ATTR of 53809 and thus, an amyloid selectivity ($EC_{50(ATTR/TTR)}$ quotient) of 15.8; see Fig. 3C and Table 1. Serum obtained from mice after immunization with the peptide/immunogen TTR34-54cyc showed a serum reactivity (EC_{50})

towards TTR of 7785 and towards ATTR of 33587 and thus, an amyloid selectivity ($EC_{50(ATTR/TTR)}$ quotient) of 4.3; see Fig. 3D and Table 1.

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Table 1: Serum reactivity and amyloid selectivity after administration of the immunogens

Immunogens	Serum reactivity (EC_{50})		**Amyloid selectivity**
	TTR	**ATTR**	
39.50	1038	15271	14.7
39.50cyc	1813	24343	13.4
34.54	3400	53809	15.8
34.54cyc	7785	33587	4.3

This was also confirmed by Fig. 4 in which the results of an ELISA assay using a serum dilution of 1:23'300 are visualized. In particular, it was shown that the serum reactivity of serum obtained from BalbC mice after immunization with the TTR peptides TTR39-50, TTR39-50cyc, TTR34-54, and TTR34-54cyc was significantly higher towards ATTR than towards TTR (as indicated by the respective OD_{450} values). Furthermore, the highest antibody titer was detected in serum obtained from mice that have been immunized with the linear peptide TTR34-54.

In summary, immunization of mice with the above-indicated TTR peptides including a carrier protein, here BSA, both with linear and cyclic TTR peptides triggered an immune response and led to the generation of anti-TTR antibodies presenting a high selectivity for amyloidogenic TTR.

CLAIMS

1. A vaccine comprising an immunogen comprising a peptide derived from transthyretin (TTR), which comprises a neoepitope which is selectively presented or accessible in misfolded, oligomeric and/or aggregated forms of TTR.

2. The vaccine of claim 1, wherein the TTR peptide is formulated with an immunogenicity enhancing agent.

3. The vaccine of claim 1 or 2, wherein the TTR peptide and neoepitope, respectively, consists of a wild type (wt) amino acid sequence of TTR.

4. The vaccine of any one of claims 1 to 3, wherein the TTR peptide comprises at least 4 amino acid residues of the TTR amino acid sequence from Lys35 to Gly57.

5. The vaccine of any one of claims 1 to 4, wherein the TTR peptide comprises the amino acid sequence WEPFA (SEQ ID NO: 1).

6. The vaccine of any one of claims 1 to 5, wherein the TTR peptide comprises or consists of at least 5, preferably at least 10, more preferably at least 15, most preferably at least 20, 21, 22, 23, 24, or 25 amino acid residues of the TTR protein.

7. The vaccine of any one of claims 1 to 6, wherein the TTR peptide has an amino acid sequence set forth in SEQ ID NO: 2, or SEQ ID NO: 3.

8. The vaccine of any one of claims 1 to 7, wherein the TTR peptide comprises a linker covalently coupled to the peptide N-terminus residue and the C-terminus residue to form a cyclic compound.

9. The vaccine of claim 8, wherein the linker comprises or consists of 1-8 amino acids and/or one or more functionalizable moieties, preferably wherein the linker amino acids are selected from glycine (G) or alanine (A) and/or wherein the functionalizable moiety is cysteine (C), most preferably wherein the linker comprises or consists of GCGGG (SEQ ID NO: 25) or GGGCG (SEQ ID NO: 26).

10. The vaccine of any one of claims 1 to 9, wherein the TTR peptide has an amino acid sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 5.

11. The vaccine of any one of claims 1 to 10, wherein the immunogenicity enhancing agent is a carrier protein, and wherein the TTR peptide is coupled to the carrier protein.

12. The vaccine of claim 11, wherein the carrier protein is bovine serum albumin (BSA).

13. The vaccine of any one of claims 1 to 11, wherein the immunogenicity enhancing agent is a heterologous T helper cell epitope, which is preferably linked to the peptide through a heterologous spacer.

14. A nucleic acid, encoding the immunogen of the vaccine of any one of claims 1 to 13.

15. An expression vector capable of expressing a nucleic acid of claim 14.

16. A host cell comprising a nucleic acid of claim 14 or an expression vector of claim 15.

17. A nucleic acid of claim 14 or an expression vector of claim 15 for producing the immunogen in a cell.

18. A composition comprising the vaccine of any one of claims 1 to 13 and an adjuvant.

19. A kit or composition comprising a nucleic acid of claim 14 or the expression vector of claim 15, and optionally an immunogenicity enhancing agent and/or at least one adjuvant.

20. A kit comprising the vaccine of any one of claims 1 to 13, or the composition of claim 18 or 19, and optionally means and/or instructions for administration of the vaccine.

21. The kit of claim 19 or 20, wherein the vaccine or the composition is present in a prefilled vial or syringe.

22. The vaccine of any one of claims 1 to 13, the kit of claim 20 or 21, or the composition of claim 18 or 19 for use in a method of treating or preventing TTR amyloidosis (ATTR) in a subject.

23. The vaccine of any one of claims 1 to 13 or 22, the kit of any one of claims 20 to 21, or the composition of claim 18 or 19 for use in a method of treating or preventing a musculoskeletal disorder or condition in a subject.

24. The vaccine or kit or composition for use according to claim 22 or 23, wherein the subject has sporadic, wild-type transthyretin-mediated amyloidosis with cardiomyopathy (ATTRwt-CM) and/or wherein the subject has a negative genetic testing for a TTR mutation.

25. A method of treating or preventing a TTR amyloidosis (ATTR), the method comprising administering the vaccine of any one of claims 1 to 13, or the composition of claim 18 or 19 to a subject in need thereof.

26. A method of treating or preventing a musculoskeletal disorder or condition, the method comprising administering the vaccine of any one of claims 1 to 13, or the composition of claim 18 or 19 to a subject in need thereof.

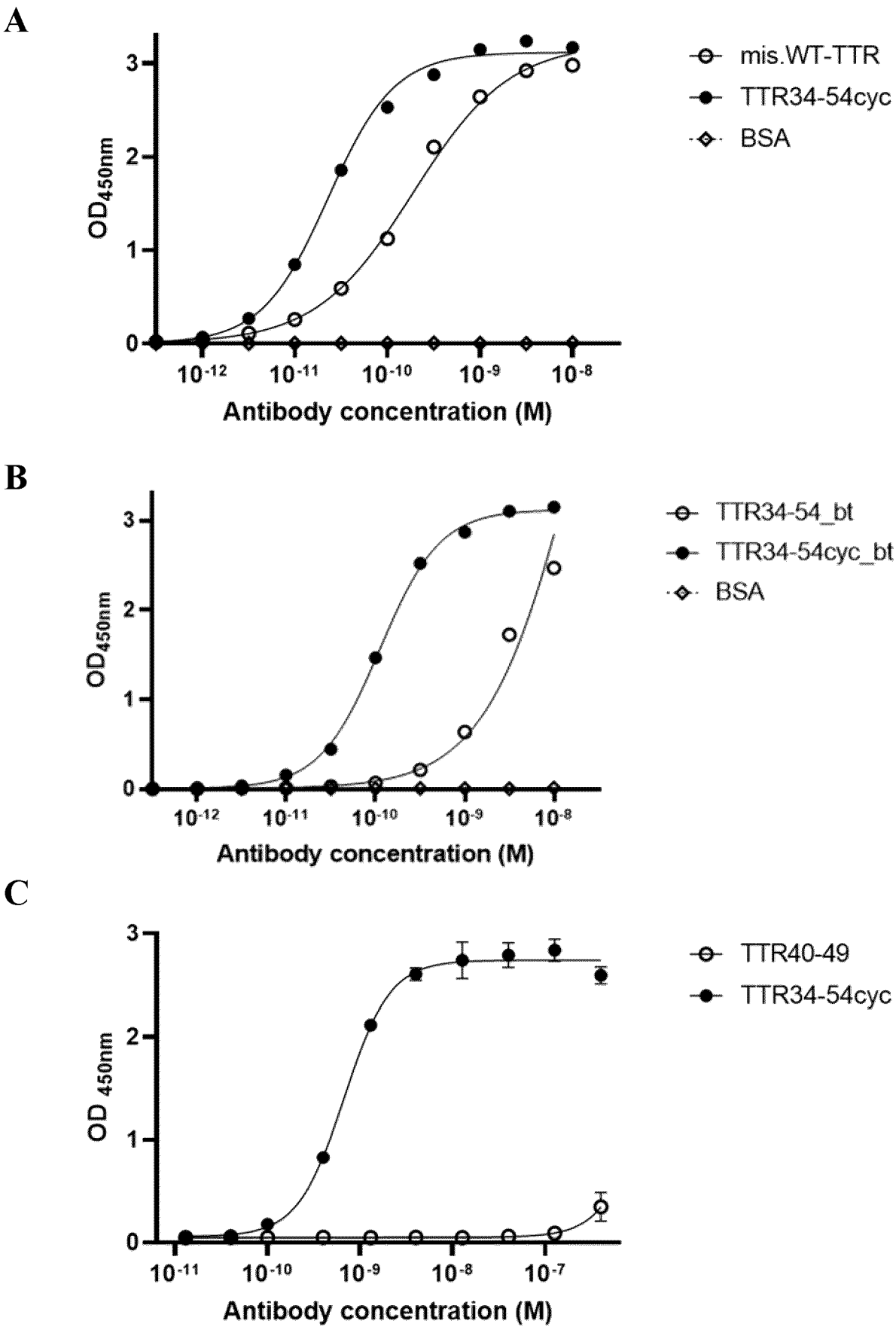
27. The method of claim 25 or 26, wherein the subject has sporadic, wild-type transthyretin-mediated amyloidosis with cardiomyopathy (ATTRwt-CM) and/or wherein the subject has a negative genetic testing for a TTR mutation.

28. The use of the vaccine of any one of claims 1 to 13, or the composition of claim 18 or 19 for the manufacture of a medicament for the treatment or prevention of a TTR amyloidosis (ATTR) in a subject.

29. The use of the vaccine of any one of claims 1 to 13, or the composition of claim 18 or 19 for the manufacture of a medicament for the treatment or prevention of a musculoskeletal disorder or condition in a subject.

30. The use of claim 28 or 29, wherein the subject has sporadic, wild-type transthyretin-mediated amyloidosis with cardiomyopathy (ATTRwt-CM) and/or wherein the subject has a negative genetic testing for a TTR mutation.

5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mis.WT-TTR
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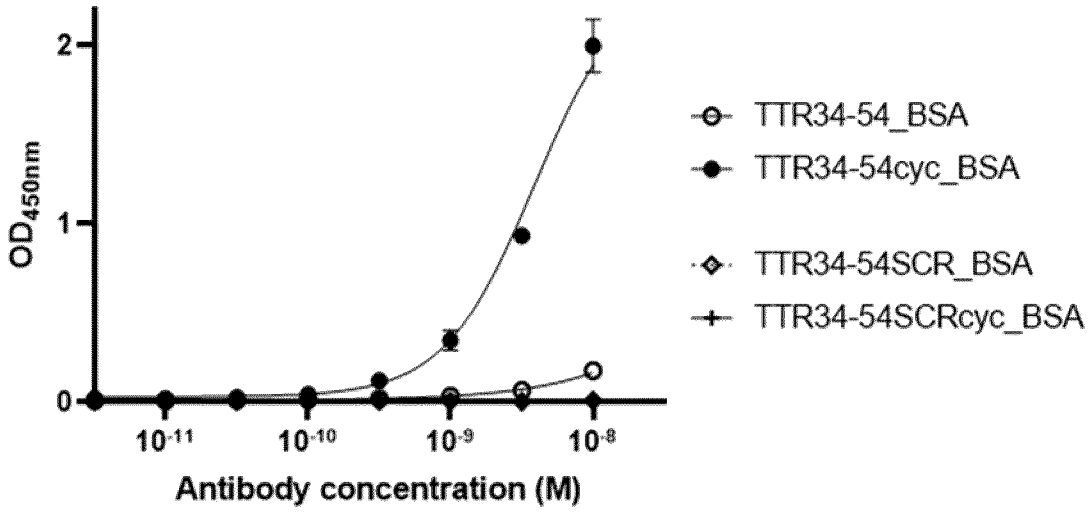
BSA
B
OD450nm
Antibody concentration (M)
TTR34-54_bt
TTR34-54cyc_bt
BSA
C
OD 450nm
Antibody concentration (M)
TTR40-49
TTR34-54cyc

Fig. 1

A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10-10
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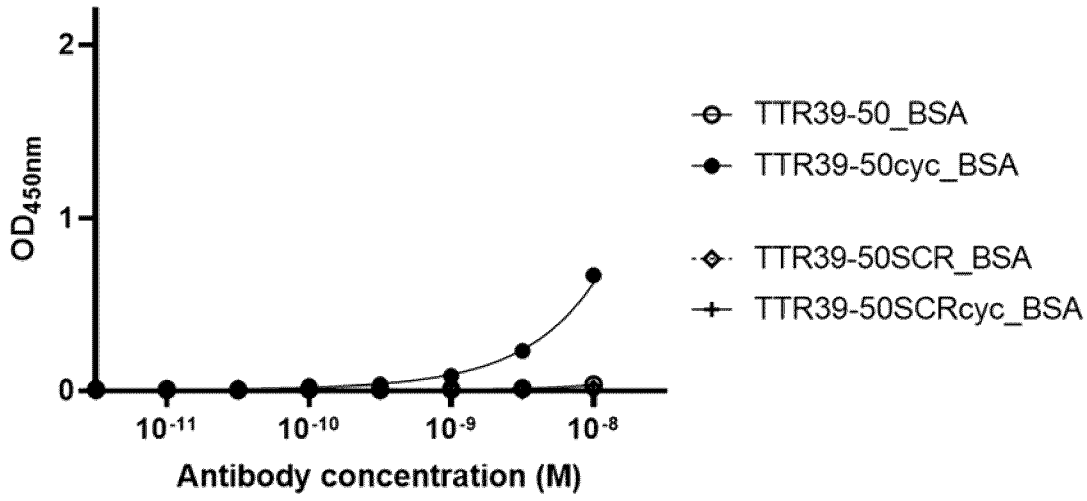
TTR34-54SCR_BSA
TTR34-54SCRcyc_BSA

B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0
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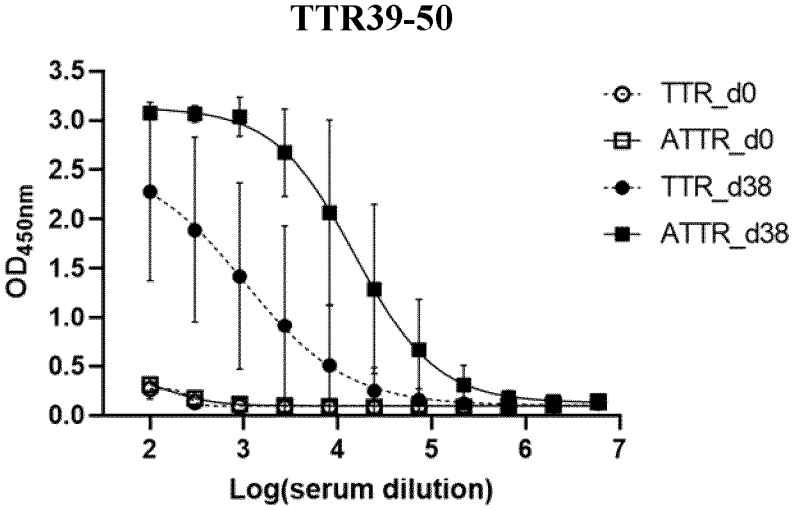
TTR39-50SCRcyc_BSA

Fig. 2

A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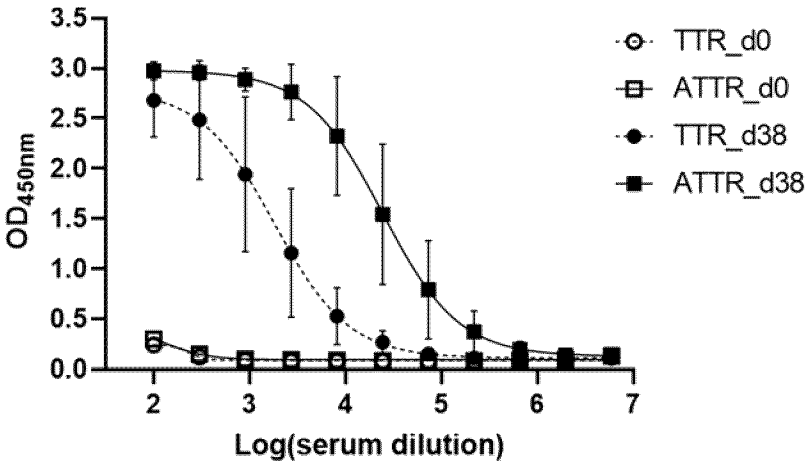
ATTR_d0
TTR_d38
ATTR_d38
OD450nm
Log(serum dilution)

B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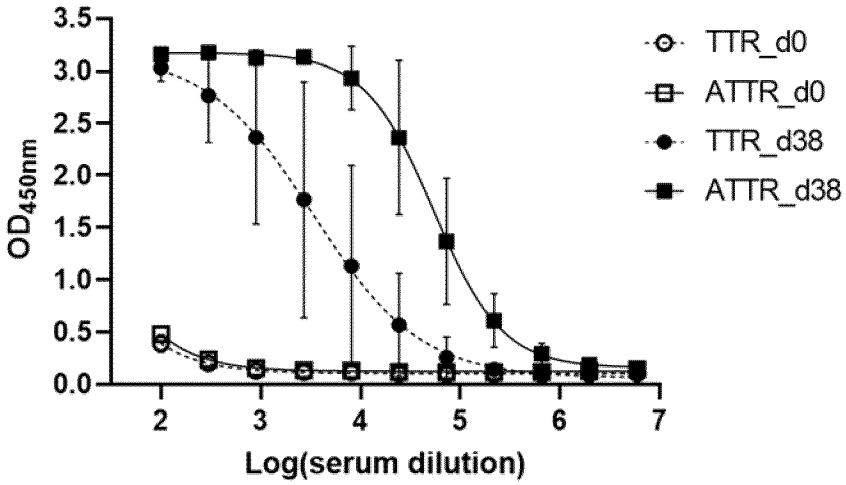
ATTR_d0
TTR_d38
ATTR_d38
OD450nm
Log(serum dilution)

C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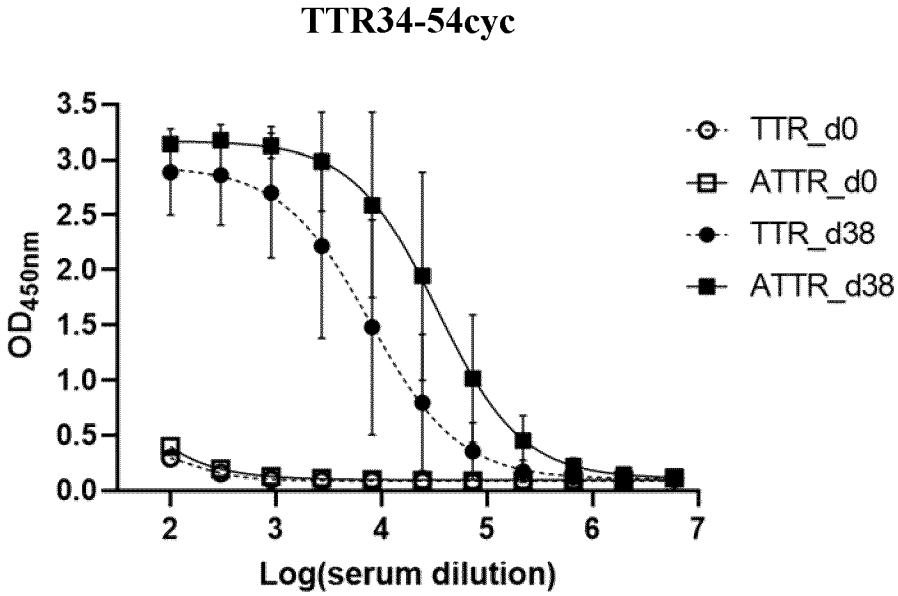
ATTR_d0
TTR_d38
ATTR_d38
OD450nm
Log(serum dilution)

Fig. 3

D



E

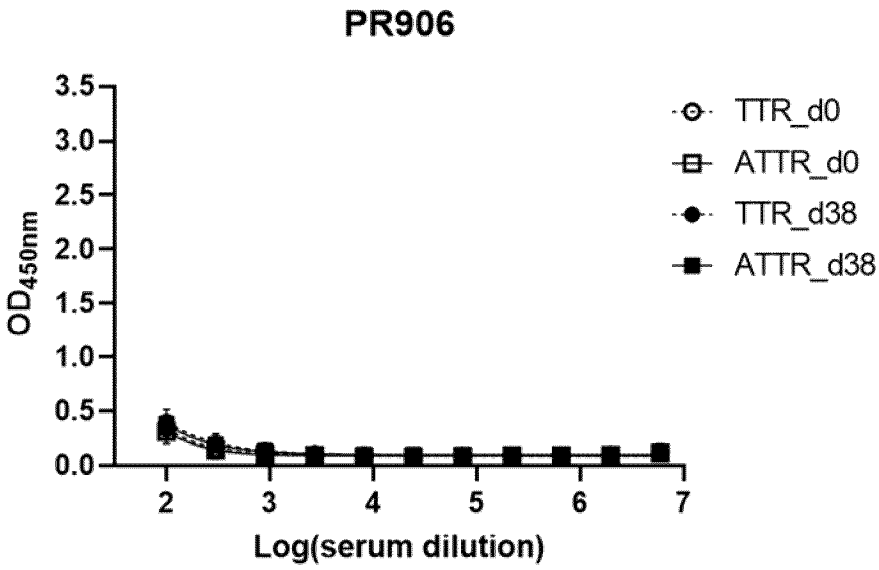


Fig. 3 (continued)

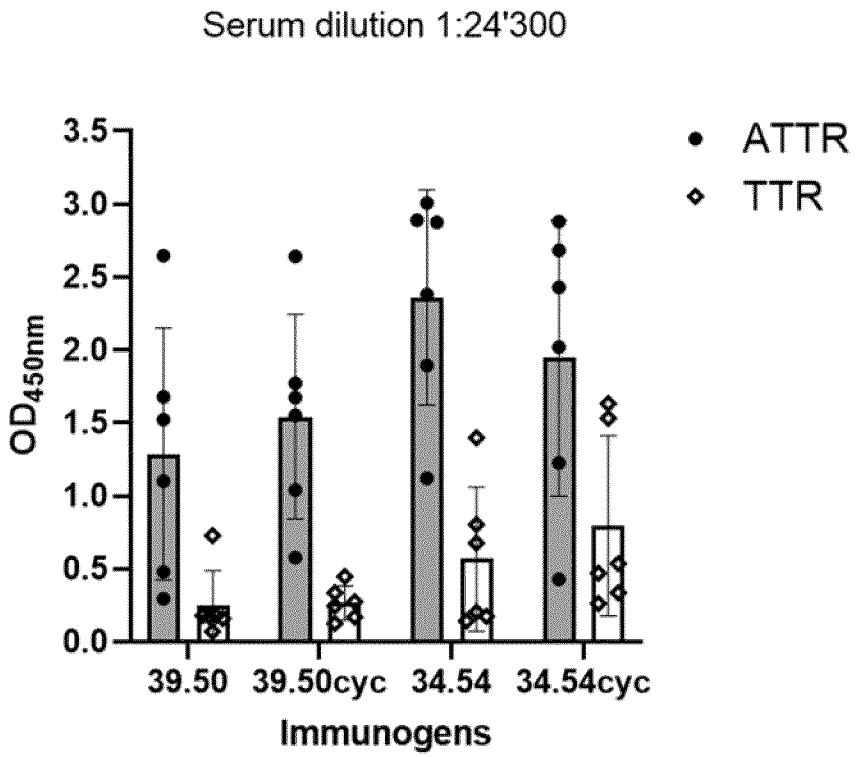

Serum dilution 1:24'300
ATTR
TTR
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
OD450nm
39.50
39.50cyc
34.54
34.54cyc
Immunogens

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/065817

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 A61P25/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JEFFREY N. HIGAKI ET AL: "Novel conformation-specific monoclonal antibodies against amyloidogenic forms of transthyretin", AMYLOID, vol. 23, no. 2, 16 March 2016 (2016-03-16) , pages 86-97, XP055372418, GB ISSN: 1350-6129, DOI: 10.3109/13506129.2016.1148025	1-3,6,9, 11,12, 14-21
Y	The introduction; The paragraph titled "Antibody generation and hybridoma selection" in the Methods section; figure 1	4,5,7,8, 10,13, 22-30

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2024	13/09/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Marinoni J-C

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2015/092077 A1 (NEURIMMUNE HOLDING AG [CH]) 25 June 2015 (2015-06-25) page 44, lines 20-23; page 108, lines 9-15; -----	4,5,7,8, 10,13, 22-30
A	MICHALON AUBIN ET AL: "A human antibody selective for transthyretin amyloid removes cardiac amyloid through phagocytic immune cells", NATURE COMMUNICATIONS , vol. 12, no. 1 25 May 2021 (2021-05-25), XP093000392, DOI: 10.1038/s41467-021-23274-x Retrieved from the Internet: URL:http://www.nature.com/articles/s41467-021-23274-x Page 2, left-hand col., lines 12-15 of the secnd paragraph -----	1-15
Y	PATRICIA Y. CHO ET AL: "A Cyclic Peptide Mimic of the [beta]-Amyloid Binding Domain on Transthyretin", ACS CHEMICAL NEUROSCIENCE, vol. 6, no. 5, 9 March 2015 (2015-03-09), pages 778-789, XP055573491, US ISSN: 1948-7193, DOI: 10.1021/cn500272a the whole document -----	8
A	LU XIAOMENG ET AL: "TANGO-Inspired Design of Anti-Amyloid Cyclic Peptides", ACS CHEMICAL NEUROSCIENCE , vol. 7, no. 9 11 July 2016 (2016-07-11), pages 1264-1274, XP093033332, US ISSN: 1948-7193, DOI: 10.1021/acschemneuro.6b00150 Retrieved from the Internet: URL:https://pubs.acs.org/doi/pdf/10.1021/acschemneuro.6b00150 -----	1-30
A	CHO PATRICIA Y. ET AL: "Transthyretin-Derived Peptides as [beta]-Amyloid Inhibitors", ACS CHEMICAL NEUROSCIENCE, vol. 5, no. 7, 9 April 2014 (2014-04-09), pages 542-551, XP093104811, US ISSN: 1948-7193, DOI: 10.1021/cn500014u ----- -/--	1-30

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/065817

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2023/099788 A1 (NEURIMMUNE AG [CH]) 8 June 2023 (2023-06-08) example 5	1,3-10, 14-17, 19,20
A	JP 2010 195710 A (UNIV KUMAMOTO) 9 September 2010 (2010-09-09)	1-30
A	MONICHAN PHAY ET AL: "Transthyretin Aggregate-Specific Antibodies Recognize Cryptic Epitopes on Patient-Derived Amyloid Fibrils", REJUVENATION RESEARCH, vol. 17, no. 2, 1 April 2014 (2014-04-01), pages 97-104, XP055256411, US ISSN: 1549-1684, DOI: 10.1089/rej.2013.1524	1-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2024/065817

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed.

   b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      ☒ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/065817

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015092077 A1	25-06-2015	AU 2014369793 A1	30-06-2016
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		PE 20210650 A1	26-03-2021
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		SI 3083681 T1	30-10-2020
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		US 2019345237 A1	14-11-2019
		US 2022144928 A1	12-05-2022
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		WO 2023099788 A1	08-06-2023
JP 2010195710 A	09-09-2010	NONE	