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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 May 2020 (14.05.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/094883 A1

(51) International Patent Classification:
*A01K 67/027* (2006.01) *C07K 14/47* (2006.01)

(21) International Application Number:
PCT/EP2019/080829

(22) International Filing Date:
11 November 2019 (11.11.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
18205502.0 09 November 2018 (09.11.2018) 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, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, 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, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, 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, 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))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) **Title:** PATIENT-DERIVED AMYLOID XENOGRAFT NON-HUMAN ANIMAL MODEL

(57) **Abstract:** Provided are a patient-derived amyloid xenograft (PDAX) non-human animal model, uses and production methods thereof as well as methods comprising the model to determine/obtain anti- amyloid drugs suitable for the treatment of an amyloidosis or amyloid-related disease and methods and processes to characterize, validate, develop and/or quality control and manufacture such drugs.

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# Patient-derived amyloid xenograft non-human animal model

## FIELD OF THE INVENTION

The present invention relates to the field of *in vivo* animal models and specifically to a patient-derived amyloid xenograft (PDAX) non-human animal model, uses thereof and methods of producing the PDAX model. The present invention also relates to methods and processes to determine/obtain, characterize, validate, develop, and/or quality control anti-amyloid drugs suitable for the treatment of an amyloidosis or amyloid-related disease as well as to the manufacture of pharmaceutical compositions thereof.

## BACKGROUND OF THE INVENTION

Amyloidoses are protein-folding diseases characterized by extracellular deposition of a specific soluble precursor protein that aggregates in the form of insoluble fibrils; see Hazenberg, Rheum. Dis. Clin. North Am. 39 (2013), 323-345 for review. These rigid and unbranching fibrils, approximately 10 nm in diameter, are characterized by a molecular β-pleated sheet structure that is usually composed of peptides arranged in an antiparallel configuration. This structure of the fibrils is responsible for its insolubility, resistance to proteolysis, and binding affinity for the amyloid-specific Congo red and thioflavine-S dyes. Three mechanisms seem to operate independently or in combination: the precursor protein may have an intrinsic propensity to misfold that becomes evident with aging (wild-type transthyretin) or at high serum levels (serum amyloid A protein and immunoglobulin free light chains); a hereditary acquired mutated protein (transthyretin); and proteolytic remodelling of the precursor protein (β-amyloid precursor protein). Interaction with the extracellular matrix also seems to be important and may be related to preferential deposition of amyloid in some organs or tissues. Extracellular deposition of amyloid fibrils in organs and tissues results in tissue infiltration and swelling leading to progressive loss off function of the affected organ.

Amyloidosis are roughly distinguished in localized forms where deposits are restricted to one organ or site of the body like Alzheimer's disease with amyloid β protein plaques in the brain or diabetes mellitus type 2 with amyloid amylin deposition in the islands of Langerhans in the pancreas, and systemic forms where fibril deposition occurs in various organs and tissues throughout the body. One of the most frequent systemic amyloidoses is amyloid transthyretin

(ATTR) amyloidosis where ATTR fibrils accumulate in multiple organs and tissues and lead to multiple organ dysfunctions with rapid disease progression and fatal consequences.

ATTR amyloidosis shows two main forms of clinical presentation, with predominant amyloid fibril accumulation in cardiac tissues leading to cardiomyopathy (ATTR-CM), and fibril accumulation in nerve fibres leading to polyneuropathy (ATTR-PN) (Ando *et al.*, Orphanet J. Rare Dis. 8:31 (2013) 1-18). TTR has an innate ability to aggregate into insoluble amyloid fibres. Amyloid misfolding occurs spontaneously at a low rate in putatively all individuals and increases with aging and senescence. It is hypothesized that in healthy subjects the immune system has the capacity to eliminate the amyloid fibrils; however, for reasons not fully elucidated yet, amyloid fibrils escape immune surveillance and accumulate up to toxic levels in ATTR patients. Regarding ATTR-CM, there is no specific treatment available yet. Usual heart failure treatments such as beta-blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers are poorly tolerated in ATTR-CM and should be avoided (Gertz *et al*, J. Am. Coll. Cardiol. 66 (2015), 2451-2466). In absence of treatment able to specifically decrease the amount of ATTR amyloid, heart transplantation is still the only available approach to restore cardiac function but with poor applicability to the elderly and fragile patient population. Liver transplantation was considered an option for mutant ATTR, but both peripheral neuropathy as well as cardiac amyloidosis can progress after liver transplantation. Tafamidis (Vyndaqel®), a transthyretin stabilizing drug, was approved in Europe in November 2011 for patients with early stage ATTR-PN. However, in a post-approval trail, neurological impairment scores worsened by 55% after 1 year of tafamidis treatment, suggesting that it could not stop disease progression (Gertz *et al*, J. Am. Coll. Cardiol. 66 (2015), 2451-2466). Similar results were obtained recently with tafamidis in ATTR-CM patients. Tafamidis reduced the speed of disease progression leading to 35% better survival and hospitalization rate after 30 months of treatment in ATTR-CM patients (Maurer *et al.*, N. Engl. J. Med. 379 (2018), 1007-1016); however, similar to what has been observed in ATTR-PN, tafamidis treatment was more beneficial to early stage patients and reduced but did not stop disease progression, and did not provide symptom regression to patients.

In the development of new drug therapies, animal models are essential in order to assess a drug's efficiency and safety. Amyloidoses drug treatments, not only in ATTR but in most if not all amyloidoses, have so far been studied using transgenic animal models, and especially transgenic mouse models, which mostly rely on mutations in respective precursor proteins

observed in human patients. For example, multiple transgenic mouse models have been presented to the scientific community as attempts to model ATTR amyloidosis, but they all suffer from major limitations including absence or rarity of ATTR amyloid deposits, high variability depending on age, gender, genetic background and housing conditions, and in all cases, absence of phenotypes mimicking patient symptoms (Takaoka *et al.*, Transgenic Research 6 (1997), 261–269; Teng *et al.*, Lab Invest. 81 (2001), 385-396; Sousa *et al.*, Am. J. Path. 161 (2002), 1935-1948; Noguchi, Exp. Anim. 51 (2002), 309-316; Panayiotou, BB reports 8 (2016), 48-54).

Analyses characterizing the transgenic mouse models indicate that these transgenic mouse models present non-amyloid TTR deposition, which does not have the characteristic tinctorial properties of amyloid, is soluble instead of being insoluble, and does not induce toxicity in mice. Accordingly, the models available are not appropriate for assessing a compound's potential to bind and clear insoluble amyloid fibrils in these models which present soluble deposits only. Accordingly, there is a need for new systems that appropriately model amyloidoses in order to enable the development of new efficient amyloidosis therapies.

## SUMMARY OF THE INVENTION

The present invention generally relates to a patient-derived amyloid xenograft (PDAX) non-human animal model. More specifically, the animal model is characterized by an implant of amyloid transthyretin (ATTR) fibrils derived from the tissue or organ of a patient suffering from an ATTR amyloidosis or ATTR-related disease.

In accordance with the present invention, the tissue is cardiac tissue, kidney tissue, liver tissue, gastro-intestinal tissue, skin tissue, muscle tissue, tongue tissue, fat tissue, salivary gland tissue, lymph node tissue, brain tissue, pancreatic tissue or any ATTR amyloidoma, wherein the amyloid fibrils are subcutaneously implanted, or implanted in the kidneys or subcapsular, in the peritoneum, the muscles, the brain, the ventricles, the nerves, the eyes, the tongue or the heart of the animal model.

In one embodiment of the present invention, the animal is a mouse, rat, *i.e.* a rodent or non-human primate, *i.e.* generally a non-human mammal.

In still a further embodiment of the present invention, the animal is non-transgenic, at least for the amyloid fibril protein.

In another aspect, the present invention relates to a method of determining and/or obtaining an anti-amyloid drug suitable for the treatment of an amyloidosis or amyloid-related disease comprising administering the drug or a variant thereof to the model of the present invention; and determining amyloid fibrils in the model, wherein the accelerated elimination or reduction of the amyloid fibrils upon administration of the drug or variant thereof compared to a control is indicative for the suitability for the anti-amyloid drug. In one embodiment, the method comprises the collection of tissue biopsies at a first and second time point after administration, and analysis and quantification of amyloid fibrils including immunohistochemistry. Preferably, the amount of amyloid fibrils is expressed as percentage of the implant tissue area, and the amyloid staining area covering the implant tissue area in the group treated with the drug or variant thereof is significantly lower than in the control group. In an additional or alternative embodiment, the accelerated elimination or reduction of the amyloid fibrils is observed in a dose dependent manner.

In one embodiment of the method of the present invention, the drug comprises an anti-amyloid fibril protein antibody or amyloid fibril-binding molecule.

In another embodiment of the present invention, the drug is an antibody and the control is a corresponding isotype antibody.

In a further embodiment of the present invention, the antibody is a human-derived, preferably human memory B cell-derived antibody and the variant thereof comprises a heterologous constant domain, preferably wherein the variant antibody is a chimeric antibody and the heterologous constant domain is derived from the same species as the animal employed in the model.

In a further embodiment of the present invention, the drug is administered intravenously, intraperitoneally, subcutaneously or orally.

In another aspect, the present invention provides a process for the manufacture of a pharmaceutical composition comprising an anti-amyloid drug and a pharmaceutically

acceptable carrier comprising subjecting the drug or a variant thereof to the method according to the present invention for determining and/or obtaining an anti-amyloid drug; and mixing the drug that has been determined as a suitable anti-amyloid drug with a pharmaceutically acceptable carrier.

In one embodiment of the present invention, the pharmaceutical composition is designed for the treatment of an amyloidosis or amyloid-related disease.

A further aspect of the present invention relates to a method for characterization, validation, development and/or quality control of an anti-amyloid drug suitable for the treatment of an amyloidosis or amyloid-related disease comprising subjecting the drug or a variant thereof to the method according to the present invention for determining and/or obtaining an anti-amyloid drug; communicating the information obtained by the method to a client, contracting party or cooperation partner and/or selecting the drug that has been determined to be a suitable anti-amyloid drug; and optionally using the anti-amyloid drug or a pharmaceutical composition comprising the anti-amyloid drug for the treatment of an amyloidosis or amyloid-related disease. Thus, one aspect of the present invention relates to the use of the model according to the present invention for drug characterization, quality control and/or development, pre- and/or co-clinical trials or selecting or validating a drug in the manufacture of a medicament for the treatment of an amyloidosis or an amyloid-related disease.

In another aspect, the present invention relates to a method of producing a patient-derived amyloid xenograft (PDAX) non-human animal model of the present invention comprising isolation of ATTR fibrils from a tissue biopsy obtained from a patient suffering from an ATTR amyloidosis or ATTR-related disease, and implantation of the isolated amyloid fibrils in a non-human animal, preferably wherein the total protein concentration is about 0.5 to 5 mg/ml, preferably about 1 to 4 mg/ml and most preferably about 2 ± 0.5 mg/ml, preferably as determined with the BCA assay.

## BRIEF DESCRITION OF THE DRAWINGS

**Fig. 1:** Presence of large ATTR aggregates detected by NI-301.37F1 in amyloid fibril extracts prepared from human heart tissues. **A** Semi-native SDS-PAGE and Western blot analysis with NI-301.37F1 of tetrameric WT-TTR (100 ng, not detected), misfolded WT-TTR (100 ng), and 0.2, 2.0 and 20 µg per lane of ATTR fibril extract. **B** Western

blot with antibody NI-301.37F1 (10nM) binding to ATTR fibrils extracted from post-mortem cardiac tissues obtained from 4 different patients with ATTR amyloidosis (sample numbers 4, 67, 87 and 70) with 30 s exposure time. Binding to sample 70 is very faint and consistent with the very low amount of ATTR detect by IHC in this tissue. NI-301.37F1 did not bind to the same protein fraction prepared from human heart tissues without ATTR amyloidosis (samples 40, 18, 60 and 48), and from one sample with amyloidosis without TTR (A+TTR- sample 94).

**Fig. 2:** Characterization of subcutaneous ATTR fibril implants in mice. **A** 24 hours after implantation, fibril grafts were recognizable by their characteristic shape and morphology different from neighbouring skin tissue. Fibril grafts were Congo red positive, and stained strongly by IHC with the human TTR antibody Dako A0002, indicating presence of ATTR fibrils. The fibril grafts were infiltrated with cells which were negative for the usual murine macrophage markers CD68, F4/80 and Iba1. **B** Fibril implants stained positive by IHC with CD11b antibody indicating that the cells infiltrating the ATTR implant and removing fibrils are neutrophils.

**Fig. 3:** The ATTR-specific antibody NI-301.37F1 binds patient-derived ATTR fibrils in a dose-dependent manner *in vivo*. **A** 48 hours after fibril implantation s.c. and antibody injection i.v., fluorescently-labelled NI-301.37F1 (NI-301.37F1-VT680) accumulated in a dose-dependent manner on the ATTR fibril implants detected by thioflavine-S fluorescence. This was not observed with the isotype antibody (Isotype-VT680). Representative images, with n=5 mice per dose level. **B** Linear correlation was observed between antibody concentration in plasma and antibody density on ATTR fibril graft *in vivo*. Dose-dependent increases on NI-301.37F1-VT680 concentration in plasma (upper left panel) and NI-301.37F1-VT680 fluorescence intensity at the fibril craft (upper right panel) across the dose range 0.05-15 mg/kg have been shown. Linear correlation between NI-301.37F1-VT680 concentrations and fluorescence intensity across the dose range 0.5-15 mg/kg (lower panel). Dotted line: linear fit ±95%CI, $R^2$=0.846, n=5 per dose level.

**Fig. 4:** Treatment with mouse chimeric NI-301.37F1 accelerates ATTR fibril clearance *in vivo*. Quantification by IHC of human TTR remaining in ATTR fibril implants 6 and 96 hours after grafting and treatment administration. Administration of ch.NI-301.37F1 at 5 mg/kg i.v. accelerated fibril elimination. n=9-10 mice per group, 2-way ANOVA with Sidak's multiple comparison test: $p<0.0001$.

**Fig. 5:** Treatment with mouse chimeric NI-301.37F1 accelerates *in vivo* ATTR fibril clearance in a dose-dependent manner. ATTR fibrils quantification using TTR IHC. N=5 per group, 1-way ANOVA with Dunnett's multiple comparison test: n.s.: not significant ($p>0.05$), **: $p=0.001$, ***: $p<0.001$.

**Fig. 6:** Characterization of subcutaneous ATTR fibril implants in mice upon treatment with mouse chimeric NI-301.37F1. **A** IBA1 staining on subcutaneous ATTR fibril grafts, 6 and 96 hours after grafting and treatment with ch.NI-301.301.37F1 or isotype at 5 mg/kg i.v. Representative images from n=5 mice per treatment group and time point, with 5 non-consecutive sections per animal. **B** CD11B staining on ATTR fibril grafts, 6 and 96 hours after grafting and treatment with ch.NI-301.37F1 or isotype at 5 mg/kg i.v. Representative images from n=5 mice per treatment group and time point, with 5 non-consecutive sections per animal. **C** LY6G staining on ATTR fibril grafts, 6 and 96 hours after grafting and treatment with ch.NI-301.37F1 or isotype at 5 mg/kg i.v. Representative images from 3 different mice per treatment group and time point. Staining performed on 5 mice per group and 2 non-consecutive sections per animal.

## DETAILED DESCRITION OF THE INVENTION

The present invention relates to a patient-derived amyloid xenograft (PDAX) non-human animal model. In the absence of a suitable *in vivo* animal model for testing anti-amyloid drugs, the inventors have developed a simple but highly efficient model for this purpose which advantageously is not necessarily based on transgenic animals. The PDAX model is characterized by an implant of amyloid transthyretin (ATTR) fibrils isolated from the tissue or organ of a patient suffering from an ATTR amyloidosis or ATTR-related disease. Surprisingly, it has been found that the amyloid fibril implant is maintained in the animal for several days and is even accessible to drug treatment. In particular, as shown exemplary for a mouse implanted with ATTR fibrils in Examples 1 and 2, the amyloid fibril implant is tolerated in the laboratory animal and presents the characteristic amyloid features in tissue biopsies following histological analyses. Furthermore, as shown in Examples 3 and 4, the PDAX model has been validated with an anti-ATTR specific antibody that has been shown to actively promote clearing of ATTR fibrils *in vivo* in another mouse model. The PDAX model is therefore highly suitable for testing compounds or drugs for their suitability as anti-amyloid drugs.

Previously, Higaki *et al.* in a poster presentation at the XVIth International Symposium on Amyloidosis; March 26–29, 2018; Kumamoto, Japan, reported on an *in vivo* model of target engagement and antibody-dependent cellular phagocytosis (ADCP) by a systemically

administered, humanized antibody against misfolded TTR (mis-TTR antibody), wherein aggregates of His-tagged TTR-V30M suspended in basement membrane matrix (Matrigel) are implanted subcutaneously into mice.

However, while in principle the method of implantation could be used for generating a PDAX animal model in accordance with the present invention, the ADCP model mice of Higaki *et al. per se* does represent an appropriate animal model according the present invention and may not be suitable for the intended purpose.

For example, despite the fact that human ATTR amyloidosis is a monogenetic disease, there are large variations in its phenotype and ATTR fibril composition, for example distinct types of amyloid fibrils, type A consisting of C-terminal ATTR fragments and full-length TTR, whereas the other, type B, consists only of full-length TTR, which seems to be related to phenotypic variation for the TTR Val30-Met mutation; see, e.g., Suhr *et al.*, J. Internal Medicine 281 (2017), 337–347.

Furthermore, it is known that in various kinds of systemic amyloidosis caused by an amyloid fibril protein deposits of the amyloid fibril protein in tissue and organ co-localize and/or co-aggregate with one or more different amyloid fibril proteins. Indeed, as known in the art and for example published in Higashi *et al.,* Brain Research 1184 (2007) 284-294, tau- and a-synuclein as well as TAR-DNA binding protein 43 (TDP43) are found coexistent in the pathology in the brains of Alzheimer's disease and Dementia with Lewy bodies; see also Lee *et al.,* Trends in Neurosciences 27 (2004), 129-134.

Colocalization of Apolipoprotein AI (apoAI) with amyloid deposits in ATTR amyloidosis has been found as well as in Amyloid A (AA) amyloidosis (AA amyloidosis), immunoglobulin (Ig) lambda light chain amyloidosis (Aλ amyloidosis), Igkappa light chain amyloidosis (Aκ amyloidosis) and Abeta2M amyloidosis; see Sakata *et al.*, J. Histochem. Cytochem. 53 (2005), 237–242. Therefore, artificial *in vitro* generated TTR-V30M aggregates may not reflect the actual structure of ATTR fibrils *in vivo*, *i.e.* in a patient suffering from ATTR amyloidosis.

In addition, whether the ADCP is significant for the suitability of an anti-TTR antibody for *in vivo* therapeutic application may be questionable and remains to be proven since the antibody used in the model was a humanized mouse monoclonal antibody raised against an artificial

antigenic peptide comprising a cryptotope within the TTR sequence and hitherto has been characterized *in vitro* only; see Higaki *et al.* Amyloid 23 (2016), 86-97.

In contrast, the PDAX model of the present invention employs human patient-derived tissue and fibrils thereof, respectively, and has been validated with a conformation-specific human-derived monoclonal antibody NI-301.37F1 against TTR aggregates, which has been demonstrated to have potential for diagnostic and therapeutic use *in vivo* in transgenic mice expressing exclusively the human V30M-TTR protein and not the mouse TTR protein (FAP mice); see international application WO 2015/092077 A1 and Michalon *et al.*, Orphanet Journal of Rare Diseases 10 (2015), Suppl. 1:P39.

Previously, Yoshimura *et al.*, Bioconjugate Chem. 27 (2016), 1532−1539, described an islet amyloid model mice, established by a method of orthotopic implantation of amylin (islet amyloid polypeptide (IAPP)) aggregates, and intended for studying the development of amylin-imaging probes, in particular $^{99m}$Tc-labeled pyridyl benzofuran derivatives. In particular, orthotopic amylin aggregates were prepared *in vitro* by diluting the peptide solution and implanted into in BALB/c-nu/nu mice (8 to 10 weeks old, male) with a surgical method; see Yoshimura *et al.* (2016), *supra*, in section "Orthotopic Amylin Aggregates for Implantation", at page 1537, left column.

However, while in principle the method of implantation could be used for generating a PDAX animal model in accordance with the present invention, the islet amyloid model mice of Yoshimura *et al.* does represent an appropriate animal model for the purposes of the present invention and may not be suitable for the intended purpose. This is because, similar as the *in vivo* model of Higaki *et al.* (2018), *supra*, for ATTR the amylin aggregates prepared *in vitro* cannot be expected to (entirely) reflect the *in vivo* structure of amylin. For example, an association was observed between phosphorylated TDP43 (pTDP43) and IAPP, which was strong and significant particularly in subjects with DM and high extent of IAPP in pancreas; see Leino *et al.*, Journal of Alzheimer's Disease 59 (2017), 43–56.

Hence, one reason for the failure of most approaches so far to provide antibodies in the therapy of amyloidosis may be due to the fact that hitherto antibody candidates have been screened with recombinant amyloid proteins and fibrils thereof generated *in vitro*, which however does not take into account the actual environment and 3-dimensional architecture of the amyloid fibrils

in the diseased tissue or organ, which in addition may involve other amyloid fibril forming proteins as well.

In a preferred embodiment, the ATTR fibrils to be implanted are isolated from cardiac tissue, kidney tissue, liver tissue, gastro-intestinal tissue, skin tissue, muscle tissue, tongue tissue, fat tissue, salivary gland tissue, lymph node tissue, brain tissue, pancreatic tissue or any ATTR amyloidoma. In a preferred embodiment, the amyloid fibrils are isolated from cardiac tissue. Methods of isolating amyloid fibrils from tissue have been described before, *e.g.*, in Tennent, Methods Enzymol. 309 (1999), 26-47. Especially, in accordance with the present invention, the amyloid fibrils are isolated using a detergent-free protocol in order to preserve the fibrils' native conformation. The efficiency of the isolation can be verified by standard techniques, like semi-native SDS-PAGE followed by Western blot, known to the person skilled in the art. As described in Example 1 and shown in Fig. 1, amyloid transthyretin (ATTR) fibrils may be efficiently isolated from *post mortem*, frozen cardiac tissue

In accordance with the present invention, the amyloid fibrils are subcutaneously or subcapsularly implanted, implanted in the kidneys, the peritoneum, the muscles, the brain, the ventricles, the nerves, the eyes, the tongue, or the heart. As shown in the Examples, amyloid fibrils are conveniently subcutaneously injected in the tight of mice where they form deposits that are accessible to drug treatments and to further analyses in skin biopsies. Specifically, Example 2 shows that following histological analyses, the deposited amyloid fibrils in skin biopsies of the model according to the present invention show the characteristic features of ATTR amyloids in human biopsies, *i.e.* were Congo red positive, and stained strongly by IHC with the human TTR antibody Dako A0002 (Fig. 2A). Accordingly, in a preferred embodiment the amyloid fibrils are subcutaneously implanted.

Common model animals that are suitable for the present invention are mice, rats, *i.e.* rodents or non-human primates, *i.e.* non-human mammal in general. In a preferred embodiment of the invention, the animal is a mouse. The mouse as model organism in the purpose of the present invention advantageously is convenient and cheap to house, and has a short generation time, *i.e.* many animals may be produced in a relatively short period of time.

Although at least the mouse genome is relatively easy to manipulate, the establishment of transgenic mouse lines or other animals is always associated with costs and rather time-

consuming. Accordingly, it is highly advantageous that for the PDAX model according to the present invention wild-type animals may be used. Therefore, in one embodiment of the present invention, the animal is non-transgenic, at least for the amyloid fibril protein. Of course, if appropriate also transgenic animals may be used, *e.g.* to better reflect the actual patient's situation. For example, it is conceivable that in order to model a patient suffering from a disease related to an immune deficiency in addition to an amyloidosis, the amyloid fibrils are implanted in immunodeficient mice. This allows the analysis of anti-amyloid drugs in the complex patient system with regard to additional diseases, for example, immune deficiencies or other diseases for which transgenic animal models are available.

In a further aspect, the present invention relates to a method of determining and/or obtaining an anti-amyloid drug suitable for the treatment of an amyloidosis or amyloid-related disease. The method comprises administering the drug or a variant thereof to the model of the present invention and determining amyloid fibrils in the model, wherein the accelerated elimination or reduction of the amyloid fibrils upon administration of the drug compared to a control is indicative for the suitability for the anti-amyloid drug, and preferably the accelerated elimination or reduction is observed in a dose-dependent manner. Rather than the intended drug, a variant of the drug may be used and tested in the PDAX model of the present invention, in particular in case of a proteinaceous such as an antibody or receptor based which may be prone to elicit an immune response in the animal. For example, as illustrated in the Examples a chimeric version of the otherwise fully human antibody can be used for testing while the drug eventually manufactured will be the parent fully human antibody.

The person skilled in the art is aware of various methods of determining amyloid fibrils. As shown in detail in Examples 2 to 5, amyloid fibrils may be analyzed in tissue sections by Congo red staining, thioflavine-S staining or immunohistochemistry with an appropriate antibody. In order to determine whether a drug treatment results in accelerated elimination or reduction of the amyloid fibrils compared to a control, the amyloid fibrils are then quantified using suitable devices and processes, like automated microscopy and image analysis processes. Furthermore, as mentioned in Example 4 and 5, ATTR fibrils undergo spontaneous fibril elimination process in the model. Accordingly, acceleration of fibril elimination or reduction upon treatment with the drug to be assessed is the output for its suitability as an anti-amyloid drug. Accordingly, the method preferably comprises collecting tissue biopsies at a first and second time point after administration, and analysis and quantification of amyloid fibrils including

immunohistochemistry. In a further preferred embodiment, the amount of amyloid fibrils is expressed as percentage of the implant tissue area, and the amyloid staining area covering the implant tissue area in the group treated with the drug or variant thereof is significantly lower than in the control group.

In a preferred embodiment, the first time point is 3-10 hours, more preferably 5-8 hours, most preferably 6 hours after administration of the drug or variant thereof and the second time point is 48-156 hours, more preferably 72-120 hours, most preferably 96 hours after administration of the drug or variant thereof.

In principle, all methods for diagnosing amyloidoses in patients' biopsies available may be applied in the method of the present invention in order to determine/analyze the amyloid fibril graft of the model organism.

As shown in Examples 3-5, an ATTR-specific antibody has been shown to specifically bind the implanted amyloid fibrils and to accelerate their elimination. Accordingly, in one embodiment of the method of the present invention, the drug comprises an anti-amyloid fibril protein antibody or amyloid fibril-binding molecule. In a further embodiment, the drug is an antibody and the control is a corresponding isotype antibody. Suitable antibody candidates are known from the prior art, *e.g.* anti-transthyretin (TTR) antibodies are disclosed in WO 2015/092077 A1, WO 2014/124334 A2, WO 2018/007923 A3, WO 2016/120810 Al, US 2017/0058023 A1 and US 9,879,080 B2. Of course, it is envisioned within the present invention that especially novel antibodies or compounds in general may be applied to the method of the present invention in order to be assessed for their suitability as anti-amyloid drugs. Besides antibodies, all types of drugs including, for example small molecules may conveniently be tested by the method of the present invention for their suitability as anti-amyloid drugs.

As mentioned, most approaches so far to provide drugs in the therapy of amyloidosis employ screening of lead candidate compound with recombinant amyloid proteins and fibrils thereof generated *in vitro*, which however does not take into account the actual environment and 3-dimensional architecture of the amyloid fibrils in the diseased tissue or organ, which in addition may involve other amyloid fibril forming proteins as well.

In contrast, the PDAX animal model of the present invention enables obtaining and selection of anti-amyloid compounds, in particular antibodies which can be reasonably expected to be as specific as to selectively bind to the target amyloid protein in the patient to be treated at the desired and necessary location of the for example toxic amyloid deposits.

In a particularly preferred embodiment, the candidate antibody is a humanized, human-like or human antibody, preferably a human-derived antibody, most preferably isolated from human memory B cells, and recombinant variants thereof, which typically substantially comprise the variable heavy and light chain of the original human-derived antibody and a human constant domain which is preferably of the IgG1 or IgG4 subtype but not necessarily identical with the constant domain of the original human-derived antibody.

However, as illustrated in the Examples for testing and validation in the PDAX model of the present invention a variant of the humanized, human-like or human antibody is preferably used, which comprises a heterologous constant domain, preferably wherein the variant antibody is a chimeric antibody and the heterologous constant domain is derived from the same species as the animal employed in the model.

The person skilled in the art is aware of various routes to administer drugs to model animals. Therefore, in one embodiment of the method of the present invention the drug is administered intravenously, intraperitoneally, subcutaneously or orally. For example, the drug is intravenously injection in the tail vein of a mouse having received an implant of isolated amyloid fibrils as shown in Examples 3 and 4.

A further aspect of the present invention, relates to a process for the manufacture of a pharmaceutical composition which comprises an anti-amyloid drug or a variant thereof and a pharmaceutically acceptable carrier. In this process, the anti-amyloid drug that has been determined to be a suitable anti-amyloid drug by the method of the present invention as described, *supra*, is mixed with a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers and administration routes can be taken from corresponding literature known to the person skilled in the art. The pharmaceutical compositions of the present invention can be formulated according to methods well known in the art; see for example Remington: *The Science and Practice of Pharmacy* (2000) by the

University of Sciences in Philadelphia, ISBN 0-683-306472, *Vaccine Protocols.* 2nd Edition by Robinson *et al.*, Humana Press, Totowa, New Jersey, USA, 2003; Banga, *Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems.* 2nd Edition by Taylor and Francis. (2006), ISBN: 0-8493-1630-8. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well-known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways. Examples include administering a composition containing a pharmaceutically acceptable carrier via oral, intranasal, rectal, topical, intraperitoneal, intravenous, intramuscular, subcutaneous, subdermal, transdermal, intrathecal, and intracranial methods. Aerosol formulations such as nasal spray formulations include purified aqueous or other solutions of the active agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Pharmaceutical compositions for oral administration, such as single domain antibody molecules (*e.g.*, "nanobodies™") etc are also envisaged in the present invention. Such oral formulations may be in tablet, capsule, powder, liquid or semi-solid form. A tablet may comprise a solid carrier, such as gelatin or an adjuvant. Formulations for rectal or vaginal administration may be presented as a suppository with a suitable carrier; see also O'Hagan *et al.*, *Nature Reviews, Drug Discovery* 2(9) (2003), 727-735. Further guidance regarding formulations that are suitable for various types of administration can be found in *Remington's Pharmaceutical Sciences*, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985) and corresponding updates. For a brief review of methods for drug delivery see Langer, *Science* 249 (1990), 1527-1533.

In one embodiment of the process of the present invention, the pharmaceutical composition is designed for the treatment of an ATTR or ATTR-related disease. Thereby, the amyloidosis is characterized by the ATTR deposit in the patient, and especially the respective precursor protein.

In another aspect, the present invention relates to a method for characterization, validation, development and/or quality control of an anti-amyloid drug suitable for the treatment of an amyloidosis or amyloid-related disease. For example, an anti-amyloid drug is characterized in dose-response studies, target engagement experiments (drug binding to target), or is

characterized for the treatment mode-of-action, *e.g.* local activation of a specific immune response by the drug at the fibril graft site, or for the PK/PD relationship, *e.g.*, dose-response as a function of the drug exposure levels. Thereby, the information about the drug that has been subjected to the method of determining and/or obtaining an anti-amyloid drug of the present invention as described, *supra*, is communicated to a client, contracting party or cooperation partner. Furthermore, the drug that has been determined to be a suitable anti-amyloid drug may be selected, and optionally the anti-amyloid drug or a pharmaceutical composition comprising the anti-amyloid drug is used for the treatment of an amyloidosis or amyloid-related disease.

The present invention further relates to the use of the PDAX model for drug characterization, quality control and/or development, pre- and/or co-clinical trials or selecting or validating a drug in the manufacture of a medicament for the treatment of an amyloidosis or an amyloid-related disease. The person skilled in the art is well aware of how to use the model of the present invention in order to characterize a drug, for example in dose-response studies, or target engagement experiments (drug binding to target). Furthermore, in accordance with the present invention, the model is used for characterization of a drug's treatment mode-of-action, *e.g.* local activation of a specific immune response by the drug at the fibril graft site, or the PK/PD relationship, *e.g.* dose-response as a function of the drug exposure levels.

In this context, it is also conceivable that the PDAX model is used in the context of personalized medicine, meaning that from an individual patient, amyloid fibrils are isolated and individually analyzed for their response to an anti-amyloid drug using the model of the present invention. With such a use of the model of the present invention, it advantageously is possible to determine whether a treatment is effective in a specific patient before even treating the patient and before subjecting him/her to possible adverse effects. Accordingly, use of the model of the present invention enables a more targeted amyloidosis therapy.

In a further aspect, the present invention relates to a method of producing the patient-derived amyloid xenograft (PDAX) non-human animal model of the present invention. The method comprises the isolation of ATTR fibrils from a tissue biopsy obtained from a patient suffering from an ATTR amyloidosis or ATTR-related disease and implantation of the isolated amyloid fibrils in a non-human animal, preferably wherein the fibril preparation has a high viscosity with a total protein concentration of the amyloid fibrils of about 0.5 to 5 mg/ml, preferably about 1 to 4 mg/ml and most preferably about 2 ± 0.5 mg/ml, preferably as determined with a

bicinchoninic acid (BCA) assay; see, *e.g.* Smith *et al.*, Anal. Biochem. 150 (1985), 76–85.An example of how the model of the present invention is produced is depicted in Example 1. As described, *supra*, the tissue biopsy from which the amyloid fibrils are isolated is cardiac tissue, kidney tissue, liver tissue, gastro-intestinal tissue, skin tissue, muscle tissue, tongue tissue, fat tissue, salivary gland tissue, lymph node tissue, brain tissue, pancreatic tissue or any other tissue having an amyloidoma deposited. The person skilled in the art is aware of different methods of isolating amyloid fibrils, *e.g.* isolation methods that preserve the native conformation of the fibrils are described in detail in Tennent, Methods Enzymol. 309 (1999), 26-47. As shown in Example 1, the isolated amyloid fibrils are implanted by subcutaneous injection but other implantation forms like subcapsular implantation, implantation in the kidneys, the peritoneum, the muscles, the brain, the ventricles, the nerves, the eyes, the tongue or the heart are conceivable.

## EXAMPLES

The following Examples 1 to 5 help illustrate embodiments of the invention. It will be appreciated that the invention is not intended to be limited by the foregoing description, which is meant to help illustrate embodiments of the invention. The patient-derived amyloid xenograft (PDAX) mouse model presented in the following Examples consists in the subcutaneous implantation of patient-derived ATTR fibrils obtained from post-mortem cardiac tissues.

### Example 1: Production of a PDAX model

Isolation of ATTR fibrils

Frozen human heart samples presenting massive TTR amyloid infiltration were processed for biochemical extraction of amyloid TTR fibrils. A detergent-free protocol was used to preserve as much as possible the conformation of the fibrils (Pras *et al.*, J. Clin. Invest. 47 (1968), 924-33; Tennent, Methods Enzymol. 309 (1999), 26-47). Briefly, the procedure consisted of repeated mechanical homogenization in ice-cold TE buffer (10 mM Tris pH 8.0, 140 mM NaCl, 10 mM EDTA, 0.1% (wt/vol) $NaN_3$, Protease inhibitor cocktail) to extract and eliminate soluble proteins, followed by repeated mechanical homogenization in ice-cold pure water. This has been described to remove the calcium-dependent binding of SAP protein on amyloid fibrils allowing the suspension of amyloid fibrils in pure water (Pras *et al.* (1968), *supra*). ATTR fibrils were further purified by precipitation with NaCl and EDTA, and concentrated by centrifugation. The total protein concentration was determined with the BCA assay and was adjusted to 2 mg/ml to ensure high viscosity of the fibril preparation. Each fibril preparation

was verified by semi-native SDS-PAGE and Western blot with the ATTR fibril-specific antibody NI-301.37F1 (10 nM) using standard procedure as described hereafter. To preserve the aggregates during the analysis process, samples were mixed with loading buffer and loaded directly on the SDS-PAGE gel, omitting the usual heat denaturation step. For each sample, 10.0 µg of total protein were loaded in 4-12% bis-tris gels in MOPS buffer and ran for 40 min at 200V. Proteins were transferred to a nitrocellulose membrane by semi-dry blotting for 60 min at 20V. Membranes were blocked for 1 h in blocking buffer (2% BSA, 0.1% tween-20 in PBS buffer pH 7.4) and incubated overnight at 4°C with NI-301.37F1 antibody diluted at 10 nM in blocking buffer. Detection was performed using HRP-conjugated anti-human IgG antibody in combination with chemiluminescent substrate.

Fig. 1A shows a semi-native SDS-PAGE and Western blot analysis with NI-301.37F1 of tetrameric WT-TTR (100 ng, not detected), misfolded-aggregated WT-TTR (100 ng), and 0.2, 2.0 and 20 µg per lane of ATTR fibril extract. The tissue extraction procedure resulted in a fraction highly enriched in ATTR fibrils, which were detected by NI-301.37F1 on a semi-native SDS-PAGE gel as aggregates of high molecular weight (Fig. 1A, left panel) that were absent in the secondary antibody only control (Fig. 1A, right panel). A similar experiment was performed with ATTR fibrils prepared from post-mortem cardiac tissues obtained from 4 different donors with ATTR amyloidosis (ATTR+), 4 donors without amyloidosis (ATTR-), and one donor with amyloidosis unrelated to ATTR (A+TTR-) (Fig. 1B). NI-301.37F1 detected selectively patient-derived ATTR fibrils in all ATTR cases, including the one with very low amount of amyloid (sample 70), and did not detect the unrelated proteins present in the same tissue fraction prepared from tissues without ATTR amyloidosis.

Implantation of patient-derived fibrils

WT SKH1 female mice were briefly anesthetized with isofluorane to receive an implantation of 100 µg ATTR fibril extract by subcutaneous injection on the thigh.

Anti-TTR Antibody

The mouse chimeric antibody ch.NI-301.37F1 was generated the human-derived monoclonal antibody NI-301.37F1 disclosed in the international application WO 2015/092077 A1. The mouse chimeric variant was designed to contain the human variable domains of NI-301.37F1 in murine constant domain backbones. In particular, the amino acid sequences of the variable heavy (VH) and light (VL) chain of human-derived monoclonal antibody NI-301.37F1 are

disclosed in WO 2015/092077 A1 in Figure 1 with SEQ ID NO. 10 and 53, respectively, for the VH chain and SEQ ID NO: 12 for the VL chain, while the mouse heavy chain constant domain corresponds to Uniprot entry P01863 and the mouse light chain constant domain corresponds to Uniprot entry P01837. In brief, gene synthesis was used to produce a synthetic heavy chain gene comprising the sequence coding for the human variable heavy chain of NI-301.37F1 followed by the sequence coding for a murine IgG2a constant heavy chain (cf. sequence mur.37F1 H), and a synthetic light chain gene comprising the sequence coding for the human variable chain of NI-301.37F1 followed by the sequence coding for a murine constant kappa light chain (cf. mur.37F1 L). These 2 genes were then sub-cloned into suitable expression vectors that were used for the transfection of CHO cells. Ch.NI-301.37F1 antibody was purified from the cell culture medium using standard processes as described in WO 2015/092077 A1 including purification of the antibody by chromatography on protein A column.

**Example 2: Characterization of the PDAX model**

24 hours after implantation of patient-derived ATTR fibrils, mice were sacrificed, skin tissues including fibril implants were collected, fixed, embedded (PPFA), and cut for histological analysis with Congo red and IHC with the human TTR antibody Dako A0002 and the macrophage markers CD68, F4/80 and Iba1. Congo red staining was performed with Putchler's modifications. In brief, tissue sections were stained with Hemalum, destained in water, incubated successively in solution I (80% EtOH, 30 g/l NaCl, 0.01% NaOH) and solution II (80% EtOH, 30 g/l NaCl, 5 g/l Congo red, 0.01% NaOH) for 30 min each at room temperature (RT), cleared in 100% EtOH with and without 0.01% NaOH, and mounted. Sections were imaged with a 20x objective in bright field and polarization modes. Immunostaining was performed according to standard procedures with quenching of endogenous peroxidase activity with 3% $H_2O_2$ in methanol for 20 min at RT, incubation in blocking buffer (PBS + 5% serum (horse/goat) + 4% BSA) for 1 hour at RT, followed by incubation with primary antibody overnight at 4°C. Detection was performed using appropriate secondary antibodies (all from Jackson Immuno-research; 1:400) in combination with the Vectastain ABC kit (Vector Laboratories) and diaminobenzidine (Dako). TTR staining was performed with the TTR antibody Dako A0002 at 1:500 dilution in combination with the biotin-conjugated goat anti-rabbit IgG antibody at 1:400 dilution; CD68 antibody was used at 1:400 dilution, the F4/80 antibody at 1:200 dilution, and the IBA1 antibody at 1:750 dilution in combination with, the HRP-conjugated biotin donkey anti rabbit IgG, respectively, at 1:400 dilution in PBS.

The amyloid fibril implants were easily recognizable from the neighboring skin tissue by their shape and appearance, clearly different from the structured and complex morphology of the skin. As shown in Fig. 2A amyloid fibril implants were positive for Congo red staining and TTR IHC. The fibril implants were infiltrated with small cells which stained negative for the murine macrophage markers CD68, F4/80 and Iba1. Instead, as shown in Fig. 2B, the cells infiltrating the amyloid fibril implants stained positive for the monocyte marker CD11b (also named integrin alpha M), indicating that these cells are most certainly neutrophils, which are like macrophages able to do antibody-mediated phagocytosis. Neutrophils are the most abundant immune cell type in the blood. Fibril grafting by s.c. injection creates a microlesion with rupture of local blood capillaries and release of neutrophils which encapsulate the fibril graft, invade it and eliminate it completely in 6 to 8 days. As shown below in Example 4, this process is accelerated in presence of ATTR antibody NI-301.37F1 indicating that the antibody is able to activate the immune system *in vivo*.

**Example 3: *In vivo* compound binding to ATTR fibrils in the PDAX model**

In order to characterize the PDAX model, the *in vivo* binding of an ATTR-specific antibody to implanted patient-derived ATTR fibrils has been analyzed. WT SKH1 female mice received under brief gas anaesthesia an implantation of 100 µg patient-derived ATTR fibril by subcutaneous injection on the thigh, followed by administration of fluorescently labelled NI-301.37F1 or isotype antibodies by intravenous injection in the tail vein at 0.05, 0.5, 5.0 or 15 mg/kg. The antibody labelling with Vivotag-680 was performed according to instructions (Perkin Elmer). In brief, the Vivotag-680 ester-reactive dye was dissolved in DMSO and mixed with antibody prepared in carbonate buffer. After 2 hours of incubation in the dark, the labeled antibody was purified by dialysis overnight at 4°C in PBS buffer.

48 hours after grafting, mice were sacrificed, skin biopsies were collected, fixed, embedded (PPFA), and cut for histological analysis. Sections were rehydrated, stained with thioflavine-S, and mounted with Dapi-containing mounting medium. Slide scanning was performed using an automated fluorescent microscope at 20x magnification using Dapi, FITC and Cy5 channels.

Subcutaneous ATTR fibril implants were clearly recognizable from the neighboring skin tissue by their specific shape and appearance, and presented strong thioflavine-S fluorescence indicating presence of fibrils with preserved amyloid conformation (Fig. 3A). Binding of the fluorescently labelled antibody NI-301.37F1 increased in a dose-dependent manner on the

fibrils but not on the surrounding tissues (Fig. 3A); Fibril binding was selective for NI-301.37F1 and was not observed with the isotype antibody. NI-301.37F1 fluorescence overlapped with thioflavine-S fluorescence, indicating binding of NI-301.37F1 on the ATTR fibrils. These results indicate that NI-301.37F1 binds in a dose-dependent manner to patient-derived ATTR fibrils implanted in mice.

NI-301.37F1-VT680 fluorescence intensity at the fibril site was quantified by software-based image analysis and served as a proxy for antibody density. Fluorescence intensity increased continuously across the whole dose range tested; more specifically, there was a continued increase in fluorescence intensity indicating that there was no saturation of NI-301.37F1 binding even between the highest doses tested (5 and 15 mg/kg).

Plasma levels for chimeric NI-301.37F1 were determined with a direct TTR ELISA and a calibration curve with known concentration of antibody. In brief, 96 well microplates were coated for 1 hour at 37°C with human wild-type TTR diluted to a concentration of 10 µg/ml in PBS buffer (pH7.4). Non-specific binding sites were blocked for 1 hour at RT with a blocking buffer (2% BSA, 0.1% tween-20 in PBS buffer pH7.4). Plasma samples were diluted 1:500 in duplicates in blocking buffer. Calibration samples were prepared similarly by diluting chimeric NI-301.37F1 antibody in duplicates in blocking buffer at a concentration range from 5 pM to 5 nM. Diluted plasma samples were quantified in duplicates (total of quadruplicates per sample): Samples were incubated overnight at 4°C, and chimeric NI-301.37F1 was detected with an HRP-conjugated, anti-mouse IgG2a antibody (Jackson Immunoresearch) at 1:4000 dilution in blocking buffer, followed by measurement of HRP activity using standard procedure.

The analysis of NI-301.37F1-VT680 concentration versus fluorescence intensity on ATTR fibrils revealed a linear correlation across the dose range of 0.5-15 mg/kg. This linear correlation indicates absence of saturation of the target with antibody doses up to 15 mg/kg (Fig. 3B). The results for the dose group 0.05 mg/kg were excluded from the correlation analysis, because the antibody concentrations may have been underestimated due to matrix interference effects at very low concentrations.

**Example 4: *In vivo* ATTR fibril clearance**

Patient-derived ATTR fibrils were implanted in WT mice by subcutaneous injection, followed by administration of mouse chimeric NI-301.37F1 variant (ch.NI-301.37F1) or corresponding

isotype antibody at 5.0 mg/kg i.v. Mice were sacrificed 6 or 96 hours later, and skin biopsies were collected for histological analysis. ATTR fibrils were detected by immunohistochemistry using the commercial human TTR antibody Dako A0002. Quantification was performed using automated microscopy and image analysis processes, and the amount of ATTR fibrils was expressed as percentage of the implant tissue area.

Subcutaneous ATTR fibril implants were clearly recognizable from neighboring skin tissue by their specific shape and appearance. 6 hours after implantation and treatment administration, ATTR fibril implants were fully stained for TTR by IHC, with TTR staining covering on average 60 to 70% of the implant tissue area in both treatment groups (Fig. 4). 96 hours after fibril implantation, TTR staining area covered only 12% of the implant tissue area in the group treated with ch.NI-301.37F1, and 39% in the isotype group (Fig. 4). The difference between the two groups was statistically significant with $p<0.0001$. These results demonstrate that ATTR fibril elimination was accelerated upon treatment with ch.NI-301.37F1.

Similar experiments were conducted based on Congo red staining and thioflavine-S staining which further confirmed that treatment with ch.NI-301.37F1 accelerated ATTR fibril elimination compared to treatment with the isotype antibody.

To further characterize the activity of NI-301.37F1 *in vivo*, ATTR fibril-grafted mice received a single administration of ch.NI-301.37F1 at 0.05, 0.5, 5.0 and 50 mg/kg i.v., or isotype at 50 mg/kg. One mouse from each group was sacrificed 6 hours after drug administration for reference (t0); all other mice were sacrificed after 96 hours, and skin biopsies were collected for histological analysis as described above.

ATTR fibrils covered 86% of the graft area at t0 and decreased to 58% 4 days (96 hours) later in isotype treated mice (Fig. 5), reflecting the spontaneous fibril elimination process in this model. Fibril elimination was accelerated upon treatment with ch.NI-301.37F1 in a dose-dependent manner. Treatment effect was statistically significant at doses of 0.5 mg/kg and above (Fig. 5), but not at the very low dose of 0.05 mg/kg due to limited effect size and low number of mice per dose group. Complete fibril elimination was obtained with both 5.0 and 50 mg/kg doses.

These data confirm the activity of NI-301.37F1 *in vivo* and indicate that activation of the immune system for the elimination of ATTR fibrils occurs already at low doses.

**Example 5: Characterization of the PDAX model upon antibody treatment**

*In vivo* clearance of ATTR fibrils in the PDAX model as shown in Example 4 was further characterized by staining for the myeloid cell markers IBA1, DC11B and LY6G.

IBA1 also known as allograft inflammatory factor 1 (AIF1) is a marker for macrophages as well as activated neutrophils. ATTR graft tissues were stained for IBA1 expression by IHC. Cells present within and around the ATTR graft tissues did not present any IBA1 expression 6 hours after grafting (Fig. 6A). Similarly, IBA1 expression 96 hours after grafting was low or absent and detected only in a small fraction of the cells within the ATTR graft area.

Cluster of differentiation molecule 11B (CD11B) is also known under the names integrin alpha M (ITGAM), macrophage-1 antigen (Mac-1) or complement receptor 3 (CR3), and is expressed by monocytes and macrophages but also granulocytes and natural killer cells. ATTR graft tissues stained for CD11B expression by IHC presented CD11B positive cells within and adjacent to the graft tissues 6 hours after grafting (Fig. 6B). At this timepoint, CD11B positive cells were typically small and amoeboid, with a subset of cells presenting extended processes. 96 hours after grafting we observed a large increase in CD11B positive cells which had infiltrated the entire ATTR graft in both control antibody as well as chNI-301.37F1 treated mice.

LY6G is a GPI-anchored protein and, together with LY6C, a component of the myeloid differentiation antigen GR-1. LY6G is predominantly present on peripheral neutrophils. ATTR graft sections stained for LY6G expression presented with LY6G positive cells within and around the ATTR grafts 6 hours after grafting independent of treatment (Fig. 6C). In contrast, no LY6G expressing cells were detected 96 hours after grafting.

# CLAIMS

1. A patient-derived amyloid xenograft (PDAX) non-human animal model, wherein the animal is characterized by an implant of amyloid fibrils derived from the tissue or organ of a patient suffering from an amyloidosis or amyloid-related disease, wherein the amyloid and amyloid fibrils, respectively, comprise amyloid transthyretin (ATTR), and wherein the amyloid fibrils are subcutaneously or subcapsularly implanted or implanted in the kidney, the peritoneum, the muscles, the brain, the ventricles, the nerves, the eyes, the tongue, or the heart.

2. The model of claim 1, wherein the animal is a mouse, rat or non-human primate.

3. The model of claim 1 or 2, wherein the animal is non-transgenic, at least for the amyloid fibril protein.

4. A method of determining and/or obtaining an anti-amyloid drug suitable for the treatment of an amyloidosis or amyloid-related disease comprising
   (a) administering the drug or a variant thereof to the model of any one of claims 1 to 3; and
   (b) determining amyloid fibrils in the model, wherein the accelerated elimination or reduction of the amyloid fibrils upon administration of the drug or variant thereof compared to a control is indicative for the suitability for the anti-amyloid drug.

5. The method of claim 4, wherein the method comprises
   (i) collecting tissue biopsies at a first and second time point after administration, and
   (ii) analysis and quantification of amyloid fibrils including immunohistochemistry, preferably wherein the amount of amyloid fibrils is expressed as percentage of the implant tissue area, and the amyloid staining area covering the implant tissue area in the group treated with the drug or variant thereof is significantly lower than in the control group.

6. The method of claim 4 or 5, wherein elimination or reduction of the amyloid fibrils is observed in a dose dependent manner.

7. The method of any one of claims 4 to 6, wherein the drug comprises an anti-amyloid fibril protein antibody.

8. The method of any one of claims 4 to 7, wherein the control is a corresponding isotype antibody.

9. The method of claim 7 or 8, wherein the antibody is a human-derived, preferably human memory B cell-derived antibody and the variant thereof comprises a heterologous constant domain, preferably wherein the variant antibody is a chimeric antibody and the heterologous constant domain is derived from the same species as the animal employed in the model.

10. The method of any one of claims 7 to 9, wherein the drug is administered intravenously, intraperitoneally, subcutaneously or orally.

11. A process for the manufacture of a pharmaceutical composition comprising an anti-amyloid drug and a pharmaceutically acceptable carrier comprising
(a) subjecting the drug or a variant thereof to the method of any one of claims 4 to 10; and
(b) mixing the drug that has been determined as a suitable anti-amyloid drug with a pharmaceutically acceptable carrier.

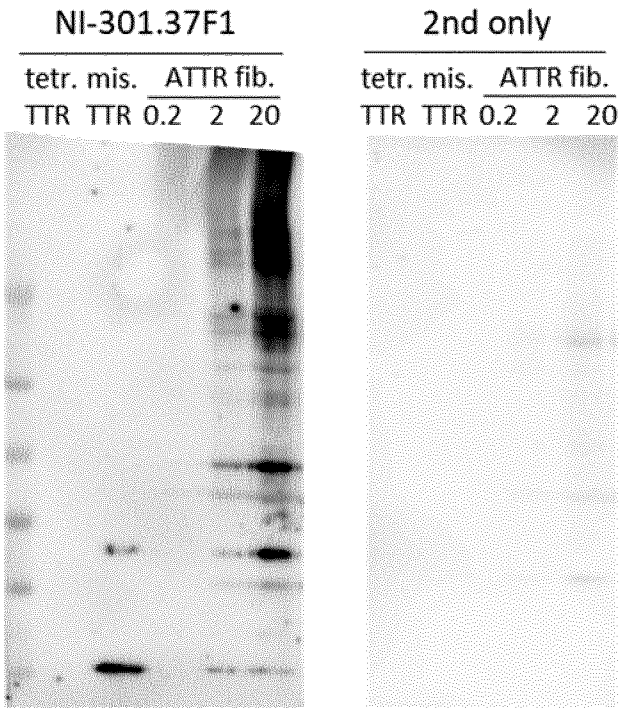
12. The process of claim 11, wherein the pharmaceutical composition is designed for the treatment of an amyloidosis or amyloid-related disease.

13. A method for characterization, validation, development and/or quality control of an anti-amyloid drug suitable for the treatment of an amyloidosis or amyloid-related disease comprising
(i) subjecting the drug or a variant thereof to the method of any one of claims 4 to 10;
(ii) communicating the information obtained in (i) to a client, contracting party or cooperation partner and/or selecting the drug that has been determined to be a suitable anti-amyloid drug; and optionally
(iii) using the anti-amyloid drug or a pharmaceutical composition comprising the anti-amyloid drug for the treatment of an amyloidosis or amyloid-related disease.

14. Use of the model of any one of claims 1 to 3 for drug characterization, quality control and/or development, pre- and/or co-clinical trials or selecting or validating a drug in the manufacture of a medicament for the treatment of an amyloidosis or an amyloid-related disease.

15. A method of producing a patient-derived amyloid xenograft (PDAX) non-human animal model of any one of claims 1 to 3 comprising
(i) isolation of amyloid fibrils from a tissue biopsy obtained from a patient suffering from an amyloidosis or amyloid-related disease, and
(ii) implantation of the isolated amyloid fibrils in a non-human animal, preferably wherein the total protein concentration of the amyloid fibrils is about 0.5 to 5 mg/ml, preferably about 1 to 4 mg/ml and most preferably about 2 ± 0.5 mg/ml.

**A**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tetr. mis. ATTR fib.
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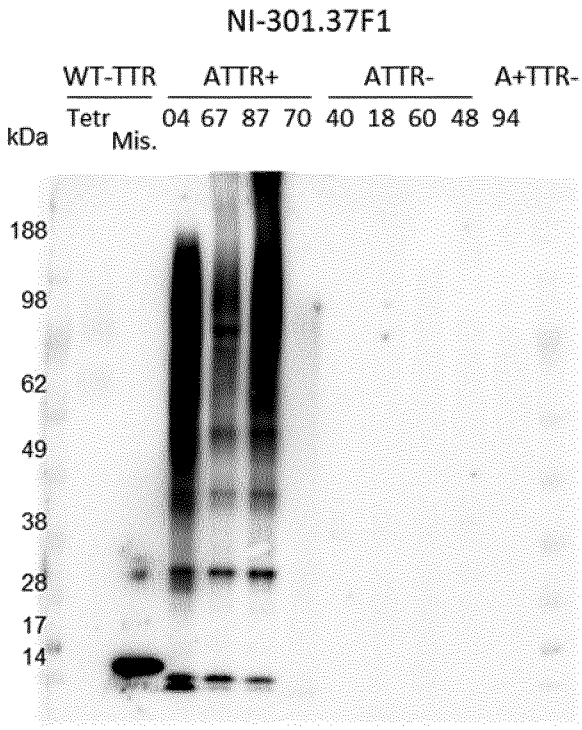
**B**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17
14

**Fig. 1**

A

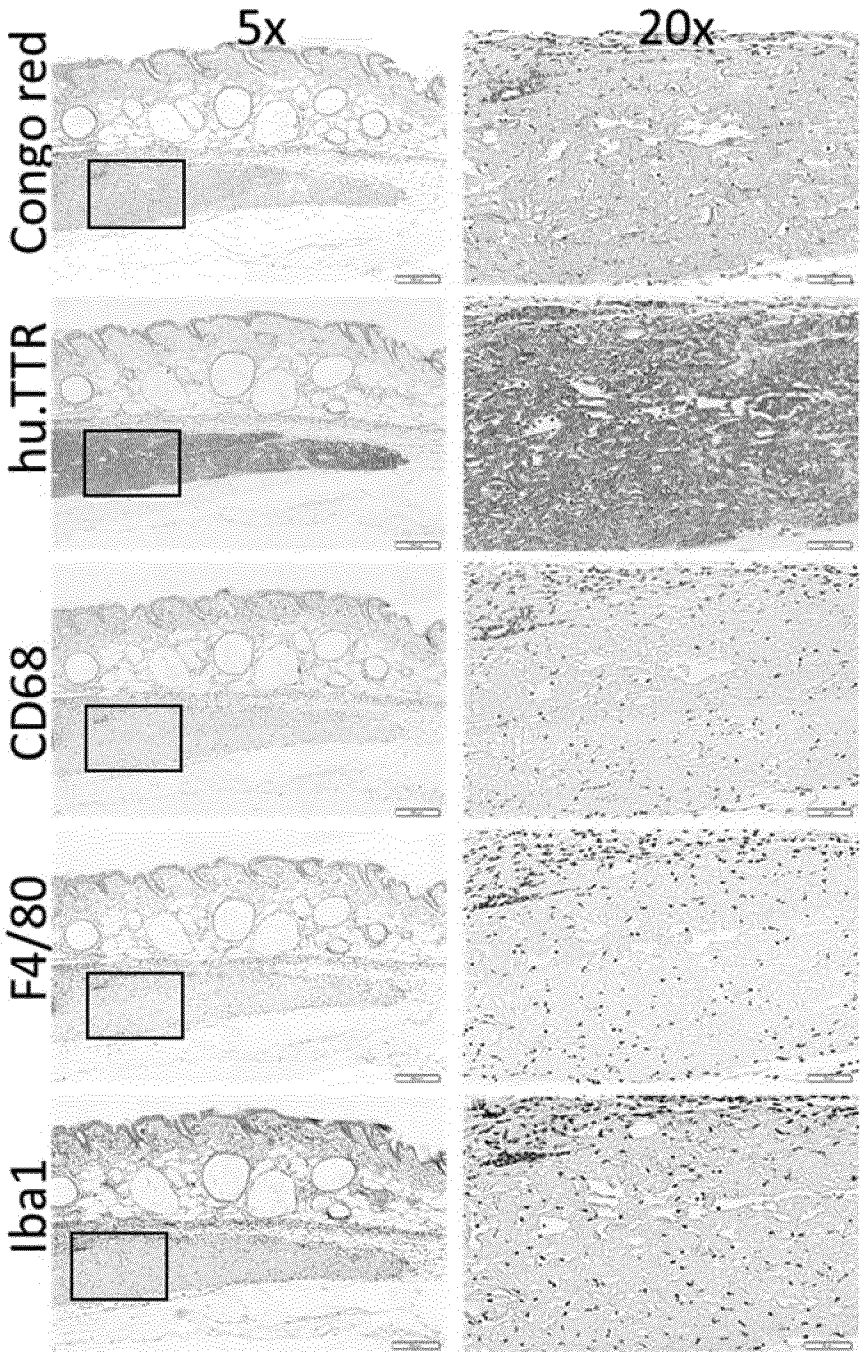



Fig. 2

**B**

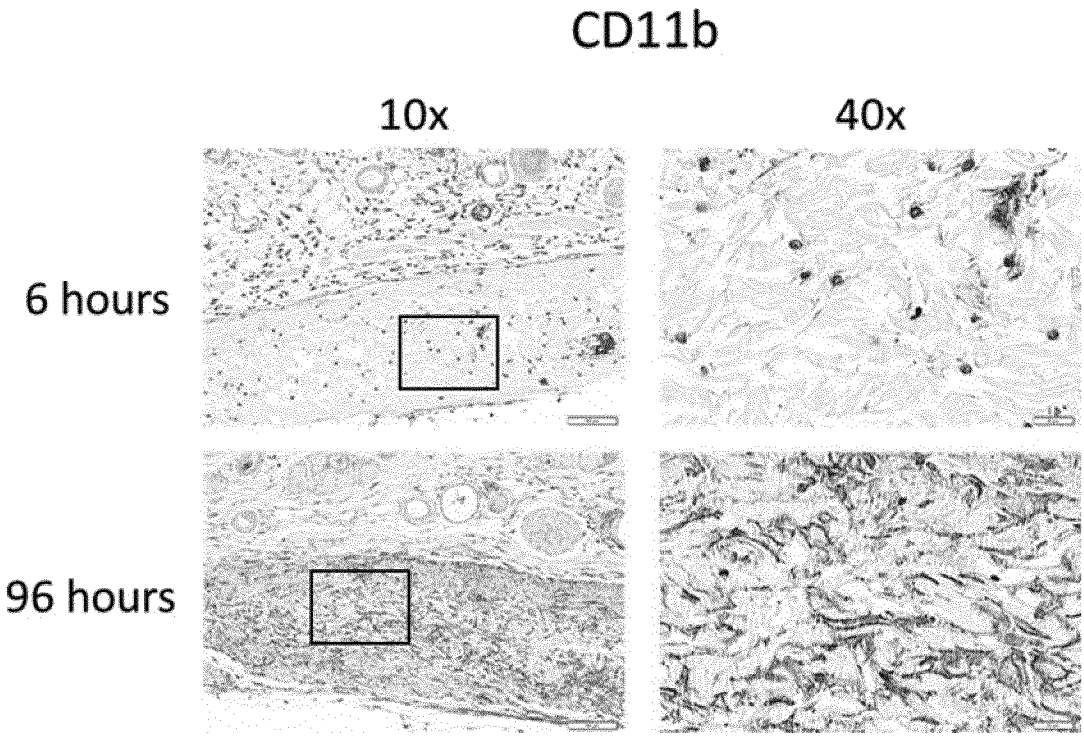
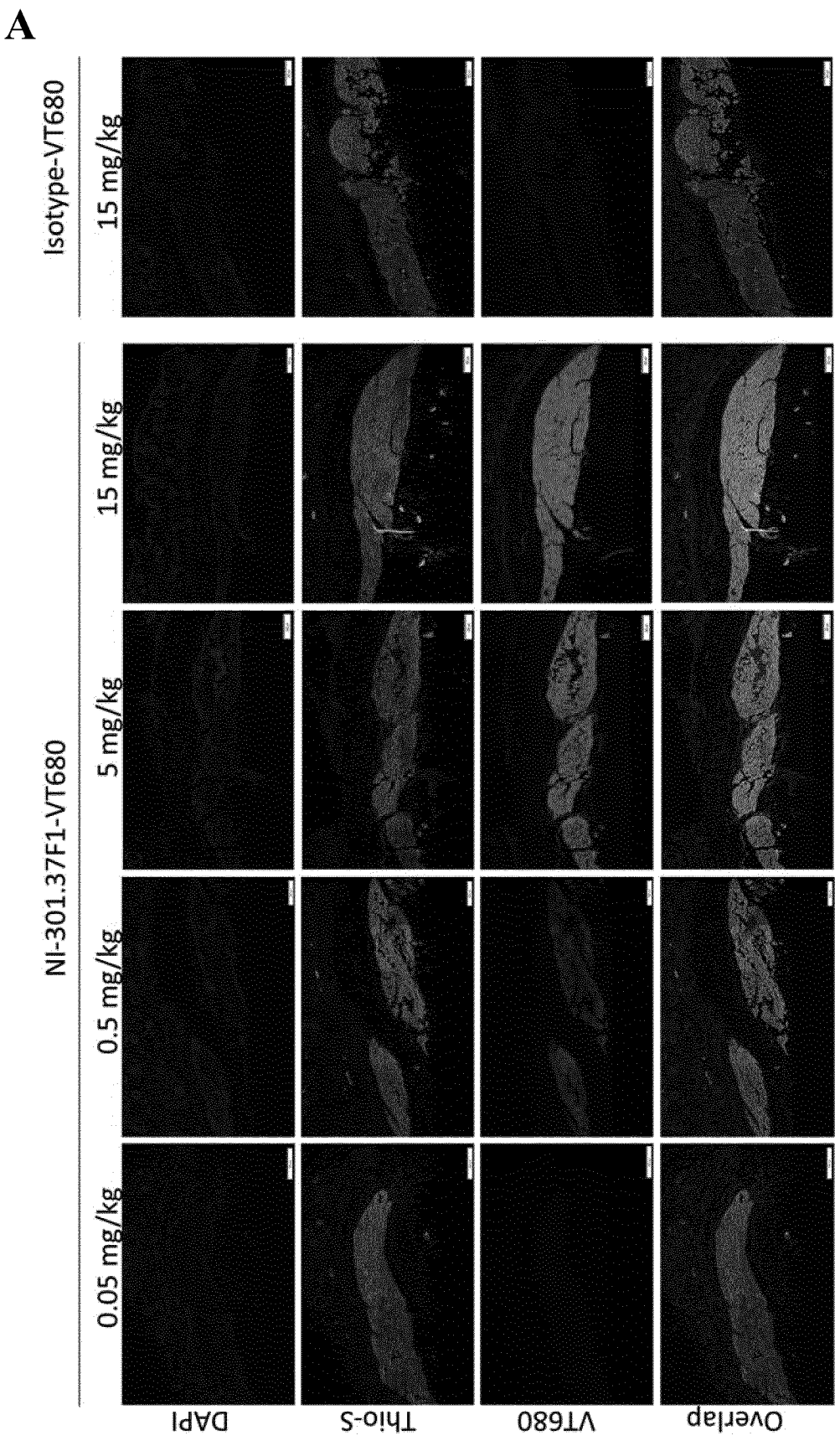



**Fig. 2 (continued)**


A
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15 mg/kg
NI-301.37F1-VT680
15 mg/kg
5 mg/kg
0.5 mg/kg
0.05 mg/kg
DAPI
Thio-S
VT680
Overlap

**Fig. 3**

**B**

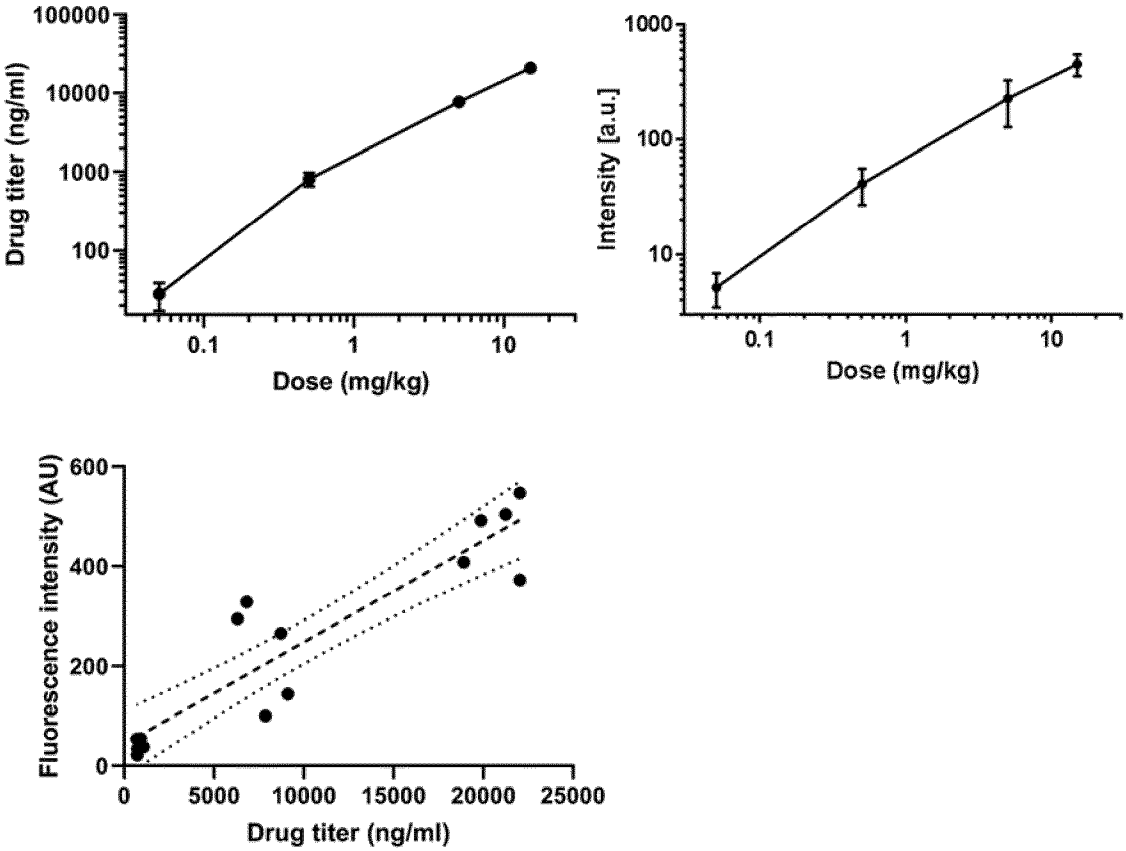


**Fig. 3 (continued)**

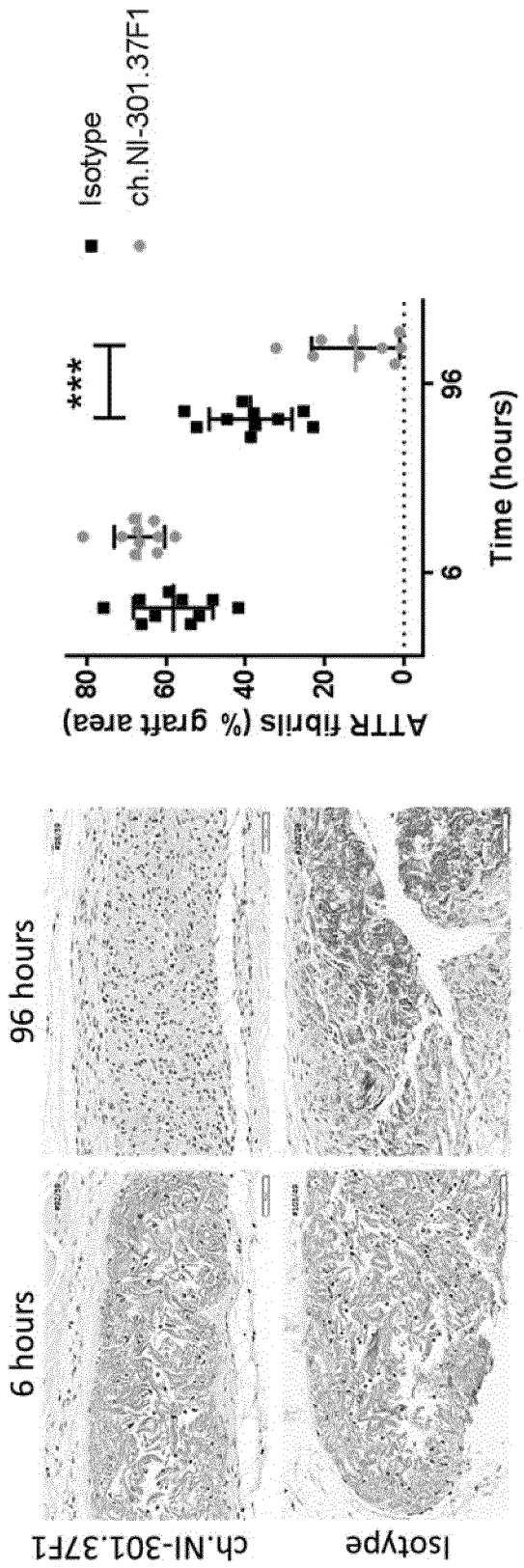
Isotype
ch.NI-301.37F1
***
ATTR fibrils (% graft area)
0
20
40
60
80
6
96
Time (hours)
96 hours
6 hours
ch.NI-301.37F1
Isotype

**Fig. 4**

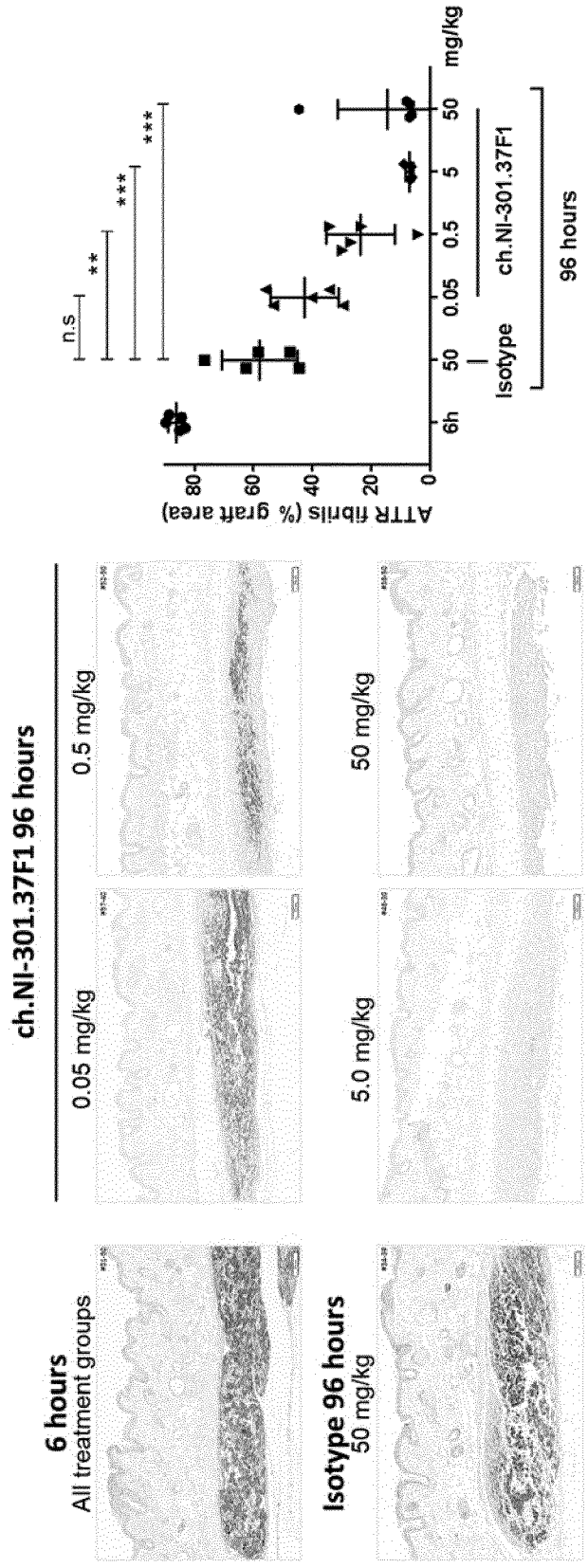
ATTR fibrils (% graft area)
0
20
40
60
80
6h
50
0.05
0.5
5
50
mg/kg
n.s
**
***
***
Isotype
ch.NI-301.37F1
96 hours
ch.NI-301.37F1 96 hours
0.05 mg/kg
0.5 mg/kg
5.0 mg/kg
50 mg/kg
6 hours
All treatment groups
Isotype 96 hours
50 mg/kg

**Fig. 5**

A

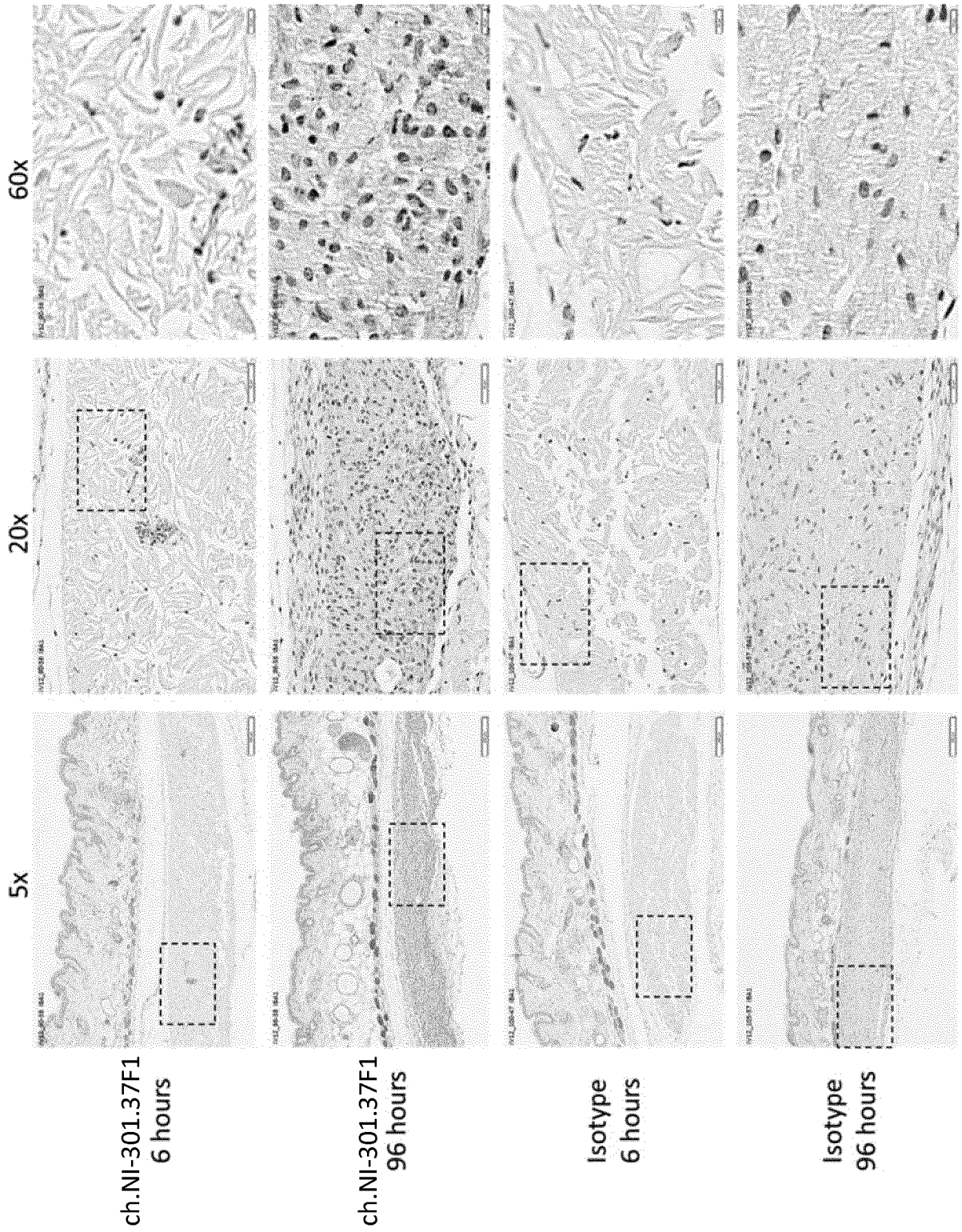
60x
20x
5x
ch.NI-301.37F1 6 hours
ch.NI-301.37F1 96 hours
Isotype 6 hours
Isotype 96 hours

**Fig. 6**

**B**

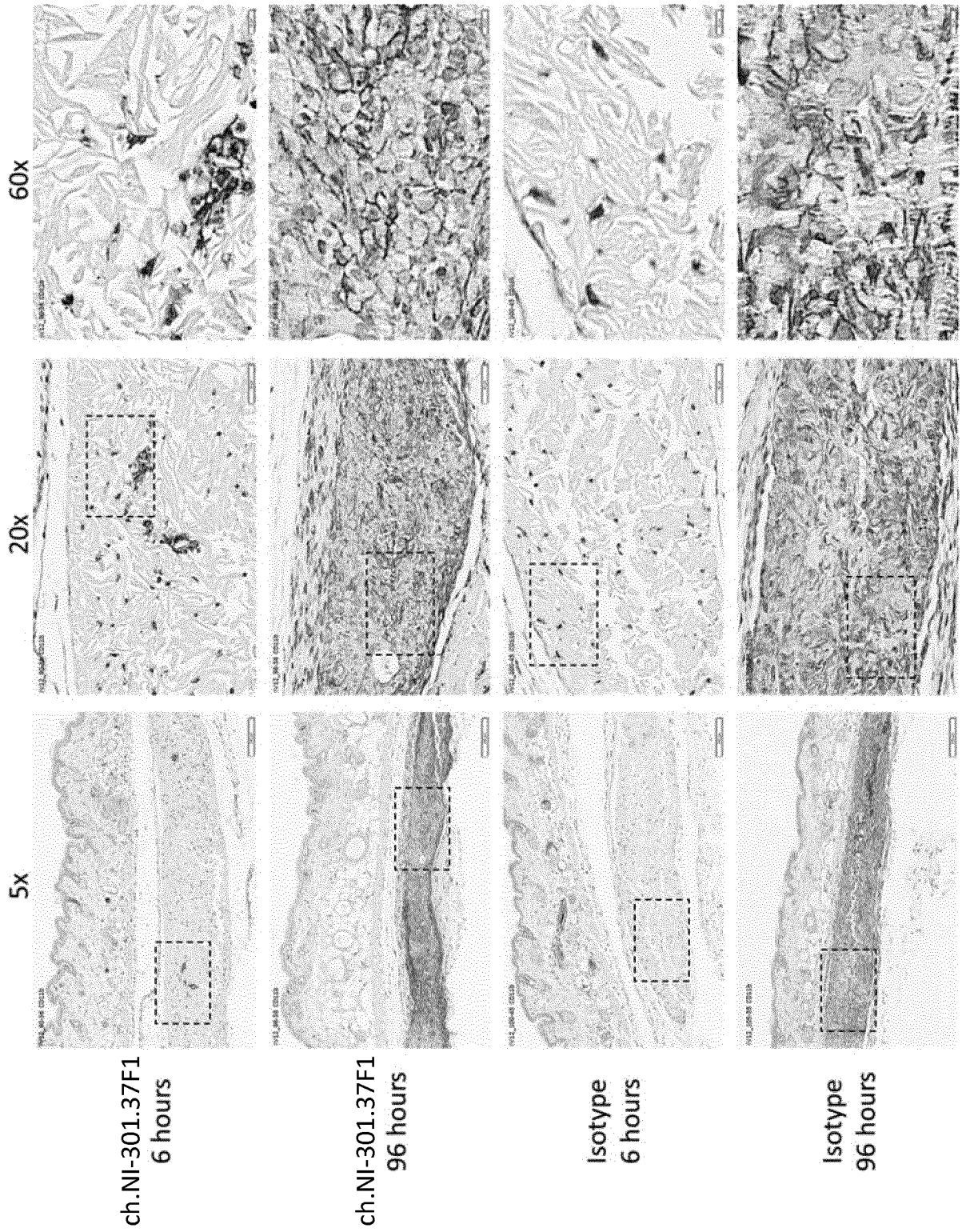


**Fig. 6 (continued)**

C

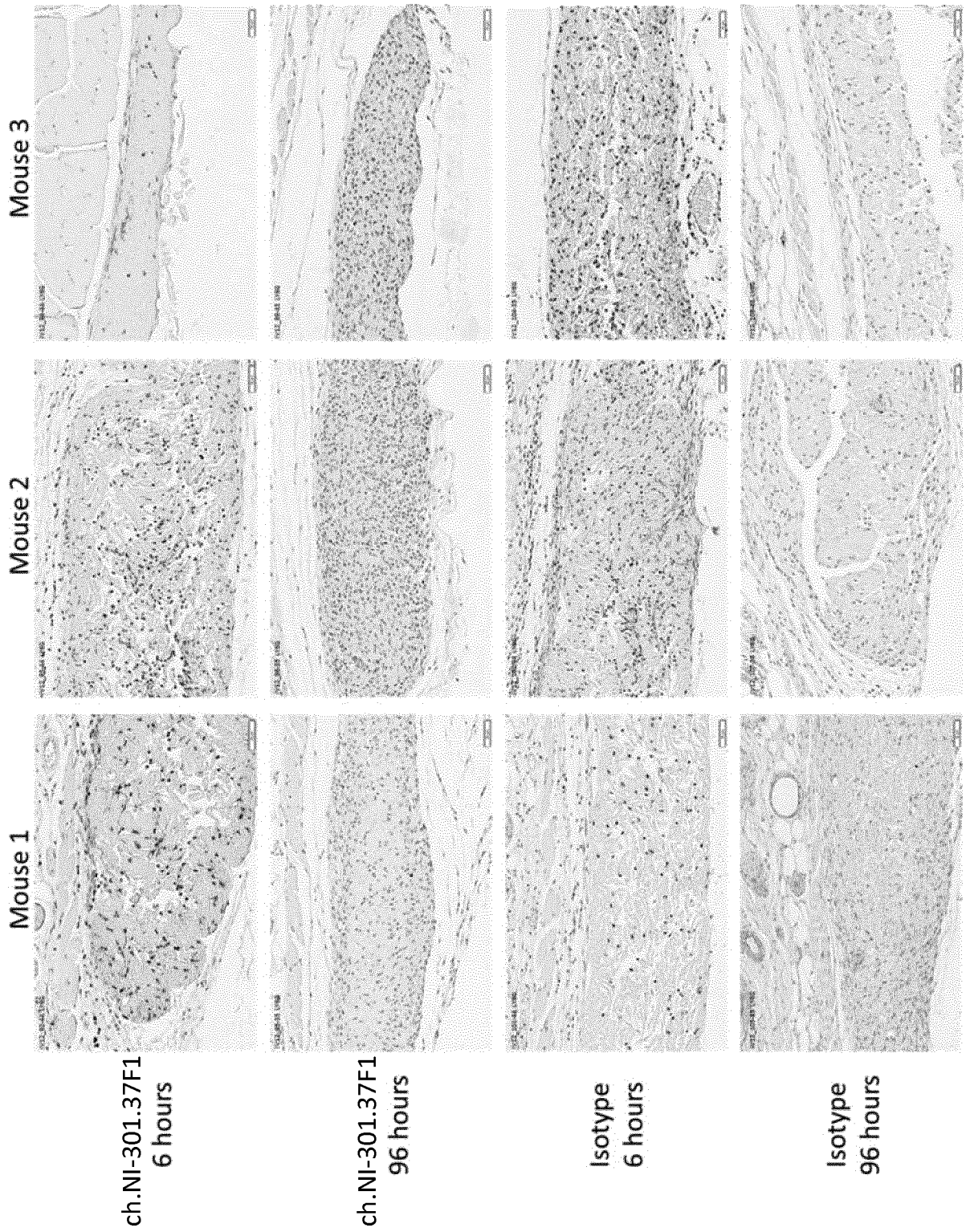


**Fig. 6 (continued)**