

UB-312, an investigational active immunotherapy targeting α -synuclein, enhances survival of a peripherally-seeded mouse model of synucleinopathy

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Additional Declarations: Competing interest reported. At the time during which this work was conducted, Justin D. Boyd and Jean-Cosme Dodart were employees of Vaxxinity, Inc., a for-profit clinical-stage biotechnology company that developed UB-312.

Abstract

Synucleinopathies are neurodegenerative diseases that exhibit pathological inclusions predominantly composed of misfolded α -synuclein (α syn) protein. Parkinson's disease, Lewy body dementia, and multiple system atrophy are the most common synucleinopathies and affect millions of people in the United States, yet there is a paucity of disease-modifying treatments. α syn-targeting active immunotherapy UB-312 was created to meet this unmet need, aimed at clearing pathological α syn and ultimately slowing disease progression. Phase I clinical trial results demonstrated good tolerability and immunogenicity of UB-312 in a Parkinson's patient population. To further evaluate the efficacy of UB-312, we performed *in vivo* experimentation in the peripherally seeded M83^{+/-} mouse model of synucleinopathy. Results demonstrate that intramuscular administration of UB-312 elicits an anti- α syn immune response and increases survivability in M83^{+/-} mice. These positive findings merit further studies investigating UB-312 as a candidate active immunotherapy for synucleinopathies.

Introduction

Pathological inclusions principally comprised of α -synuclein (α syn) protein occur in a group of neurodegenerative diseases collectively termed synucleinopathies. In Parkinson's disease (PD) and Lewy body dementia (LBD) α syn predominantly accumulates in neurons in the form of Lewy bodies and neurites¹. The discovery of many missense mutations in the α syn gene (*SNCA*) as well as *SNCA* duplication/triplication in familial PD and LBD provide a direct causal link between this spectrum of diseases and α syn pathobiology^{2,3}.

Current PD treatments are limited, with no robust FDA-approved disease-modifying therapies targeting pathological proteins available to patients, although many candidates are currently under investigation in clinical trials⁴. One such investigational therapeutic is the active immunotherapy UB-312, a synthetic α syn peptide conjugated to a T helper peptide⁵. UB-312 is designed to induce antibodies against pathological species of α syn while evading T cell-mediated toxicity commonly associated with active immunotherapies. This therapeutic approach is ultimately aimed at reducing toxic α syn species and thus ameliorating disease progression.

UB-312 has previously demonstrated positive results in the Thy1SNC/15 mouse, which expresses wildtype, human α syn in neurons, modeling a gradually progressive, early stage of α syn toxicity⁶. In that work, intramuscular (IM) administration of UB-312 in young adult mice modified motor impairments and reduced α syn oligomers in the brain 15 weeks after initiation of immunization. Additionally, a recent phase 1 clinical trial tested safety, tolerability, and immunogenicity of UB-312 in patients with PD^{7,8}. Repeated intramuscular administration of UB-312 demonstrated limited, predominantly mild, adverse events, while 12 of 13 individuals who received UB-312 demonstrated anti- α syn seroconversion. Early exploratory analyses did not show significant UB-312-associated changes in clinical scales; however, a significant reduction of α syn in cerebrospinal fluid was observed in a subset of UB-312-treated patients⁸.

Herein, we have performed *in vivo* experimentation in a more rapidly progressing mouse model of synucleinopathy, the seeded M83^{+/-} model, to further evaluate the efficacy of UB-312 in the context of a more aggressive disease timecourse. The M83 mouse overexpresses disease-associated A53T human α syn in the central nervous system (CNS), but hemizygous (M83^{+/-}) animals do not develop any pathology before 18 months of age⁹. We use a well-characterized hindlimb intramuscular inoculation of M83^{+/-} mice with human α syn preformed fibrils (PFFs) to induce a more aggressive and predictable synucleinopathy that results in terminal paralysis before 9 months of age^{10,11}. This CNS neuro-invasion model relies on neuronal retrograde transport of α syn seeds to drive the formation of robust synuclein pathology in the CNS, particularly the spinal cord, brainstem, midbrain, and thalamus. Efficacy assessment of peripheral administration of UB-312 in this context is aimed at identifying the extent to which this active immunotherapy impedes progression of an aggressive, periphery-to-CNS-seeded model of synucleinopathy in an effort to further illuminate the therapeutic potential of UB-312.

Methods

Animal Usage

Transgenic mice hemizygous for human α syn with the A53T mutation under the control of the mouse prion protein promoter (line M83^{+/-})⁸ were used for the studies. All procedures and husbandry were approved by the University of Florida Institutional Animal Care and Use Committee and performed accordingly. Experimental animals were euthanized by CO₂ inhalation followed by transcardiac perfusion.

Experimental Treatments of M83 Mice

To initiate a previously characterized¹⁰ pathological α syn neuro-invasion experimental model, 3 cohorts of 12 M83^{+/-} mice (6 male/6 female) were anesthetized with isoflurane and injected with 2 μ g of human α syn PFFs in the gastrocnemius muscle at 2 months of age. During the same treatment session, one cohort was also injected with UB-312 (50 μ l volume, full formulation described below in “Generation of UB-312”) in the gastrocnemius muscle of the opposite leg, representing the 0 timepoint for UB-312 treatment. Similar, dose-matched (50 μ l) boosts of UB-312 were injected into alternating gastrocnemii 3, 6, 12, and 15 weeks thereafter (see Fig. 1 for tabulated summary). The remaining 2 cohorts of 12 PFF-injected M83^{+/-} mice (6 male/6 female) received saline or adjuvant vehicles instead of UB-312 at all treatment time points. Serum was collected from each animal one day prior to every injection session. Animals were routinely monitored for motor impairment and euthanized upon reaching a hindlimb paralysis-based endpoint. Assessment of motor symptoms and determination of end-stage paralysis was performed by research scientists (unblinded) with the assistance of veterinarian technicians (blinded) in accordance with predetermined IACUC protocol standards.

Generation of UB-312

UB-312 is a synthetic peptide-based immunotherapy targeting α syn. The active pharmaceutical ingredient in UB-312 is a fully synthetic, 33 amino acid peptide immunogen designated p4573kb, which consists of a T-helper cell epitope covalently linked to a peptide spacer and a B-cell epitope represented by a 10-residue long fragment of the α syn protein. UB-312 drug product is comprised of p4573kb formulated with a proprietary polyanionic CpG oligonucleotide (ODN, CpG1) at a 1.8:1 (peptide:CpG1) molar charge ratio. The peptide/CpG1 complex is further formulated with alum adjuvant (Adju-Phos®). The final formulation contains 1.5 mg/ml of peptide immunogen, 445 μ g/ml of CpG1, and 1.6 mg/ml of Aluminum.

Preformed Human α -Synuclein Fibrils (PFFs) Generation

The pRK172 bacterial expression vector bearing full-length, wildtype, human α syn cDNA was used to express recombinant α syn protein in BL21(DE3)/RIL Escherichia coli (E. coli; New England Biolabs Inc.). These bacteria were centrifuged and re-suspended in high-salt buffer (0.75 M NaCl, 50 mM Tris, pH 7.4, 1 mM EDTA) with protease inhibitor cocktail and heated at 100°C for 10 min. 20,000xg centrifugation was performed for 30 minutes to remove cell debris. Supernatants were collected and dialyzed against 100 mM NaCl, 10 mM Tris, pH 7.5 and then applied to a Superdex 200 gel filtration column (Amersham Pharmacia Biotech, Inc., Piscataway, NJ) for size exclusion chromatography. Resultant fractions were assessed by SDS-polyacrylamide gel electrophoresis (PAGE) and subsequent Coomassie Blue R-250 staining to determine the presence of α syn protein. Positive fractions were pooled and concentrated with Amicon Ultra-15 units (Millipore Corp., Bedford, MA), dialyzed against 10 mM Tris, pH 7.5, applied to a Resource Q column (GE healthcare) and eluted with a 0-0.5 M NaCl gradient. α Syn protein concentration was determined by bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL) using bovine serum albumin (BSA) as a standard. Purified α Syn monomers were then shaker-incubated (1050 rpm, 37°C) on an Eppendorf Thermomixer at 5 mg/mL in sterile PBS for 48 hours to drive assembly into PFFs. α Syn fibril formation was assessed by K114 fluorometry as previously described²¹. To fragment PFFs into an array of shortened fibrils, PFFs were diluted in sterile PBS at a final concentration of 1 mg/ml and incubated in a Branson 2800 water bath sonicator at room temperature for 1 hour before being aliquoted and stored at -80°C. Each injection session was performed with a fresh aliquot of material from the same batch of sonicated PFFs to limit freeze-thaw and inter-batch variability.

Serum ELISA Against Recombinant α Syn

Submandibular blood collection was performed on all mice approximately 24 hours before each (IM) administration of UB-312, adjuvant, or saline. Blood was collected in a serum separator microtube (Beckton Dickinson) and centrifuged at 2,000g for 10 min. Following centrifugation, serum was collected, aliquoted, and stored at -80°C.

For ELISA, clear flat-bottom Nunc MaxiSorp 96-well plates were pre-coated with recombinant human α syn protein. Either PFFs or α syn monomers were diluted to a final concentration of 2 μ g/ml in carbonate-bicarbonate coating buffer and applied to plates at 100 μ l/well. Plates were then sealed and incubated overnight at 4°C. The next day, sera samples were thawed on ice. α syn protein solution was removed from 96-well plates and plates were quick-washed 4x with PBS-T on an automated plate-washer. Plates were then twice blocked with 200 μ l/well Super Blocking Buffer (ThermoFisher 37515) at 25°C, 500 rpm on a thermoshaker. The first block was performed for 30 minutes, the second for 60 minutes. Blocking Buffer was decanted and newly replenished between blocks without additional wash steps. During block steps, sera samples were serially diluted in 2% BSA in PBS and held on ice. After blocking, diluted sera samples were transferred to plates at 100 μ l/well. Plates were then incubated at 25° C for 1 hour without shaking. Following sera incubation, plates were again washed 4x with PBS on an automated plate washer. 100 μ l of detection solution (250 ng/mL recombinant protein A/G peroxidase in PBS-T containing 2% skim milk) was added to each well, and plates were incubated at 25° C, 500 rpm for 1 hour. Following this incubation, plates were washed 6x with PBS-T on a plate washer. 100 μ l of room-temperature-equilibrated TMB substrate was added to each well. Plates were incubated for exactly 5 minutes in a light-protected environment. Following incubation with 3,3',5,5'-tetramethylbenzidine reagents (ThermoFisher 1854050), reactions were quenched by adding 100 μ l/well of 2 N sulfuric acid stop solution. Absorbance was read at 450 nm on a microplate reader after a 5 second orbital shake.

Antibodies

Mouse monoclonal antibody 81A recognizes α syn phosphorylated at Ser129 (pSer129)¹². Mouse monoclonal antibody 2G5 is specific for α Syn carboxy-terminally truncated at residue 103 (x-103) and has also been previously described¹³.

Tissue Processing and Immunohistochemical Analyses of α Syn Pathology in Mice

Mice were euthanized with CO₂ and perfused with a heparin/PBS solution, as previously described.¹¹ Brains of mice were harvested and fixed in 70% EtOH/150 mM NaCl for 48 hrs. Brains were then paraffin embedded and coronally cut into 5 μ m sections. 3,3'-Diaminobenzidine (DAB) immunohistochemistry was performed with antibodies 81A and 2G5 using previously established methods²². Sections were deparaffinized and rehydrated through brief immersions in xylene and graded ethanol solutions (100%, 90%, and 70%). Heat-induced epitope retrieval (HIER) was performed in a steam bath for 30 minutes in a deionized H₂O/0.05% Tween solution or for 60 minutes in a solution of modified citrate buffer (Target Retrieval Solution Citrate pH 6; Agilent, Santa Clara, CA) for antibodies 81A or 2G5, respectively. After antigen retrieval, sections were washed in running deionized H₂O for 15 minutes. For 2G5, sections were immersed in 70% formic acid for 10 minutes and subsequently washed in running deionized H₂O for 15 minutes. To quench endogenous peroxidases, sections were immersed in a solution of 1.5% hydrogen

peroxide/0.005% Triton-X-100 diluted in PBS, pH 7.4 (Invitrogen, Waltham, MA) for 15 minutes, followed by three 5-minute washes with agitation in 0.1M Tris, pH 7.6. Sections were then blocked with a 2% fetal bovine serum (FBS)/0.1M Tris, pH 7.6 solution for 10 minutes. Slides were incubated in primary antibodies in blocking solution, 1:3000 for 81A or 1:1000 for 2G5, and incubated overnight at 4°C. The next day, primary antibody was removed through three 5 minute washes with agitation in 0.1M Tris, pH 7.6. Sections were then blocked with a 2% FBS/0.1M Tris, pH 7.6 solution for 10 minutes. Anti-mouse biotinylated IgG (Vector Laboratories, Burlingame, CA) diluted 1:3000 with FBS/0.1M Tris, pH 7.6 was mixed 10:1 with ImmPRESS polymer anti-mouse IgG reagent (Vector Laboratories, Burlingame, CA) and applied as a secondary antibody for 60 minutes. The secondary antibody was removed with three 5-minute washes with agitation in 0.1M Tris, pH 7.6. Sections were then incubated with an avidin-biotin complex (ABC) solution (Vectastain ABC Elite kit; Vector Laboratories, Burlingame, CA) for 60 minutes followed by three 5-minute washes with agitation in 0.1M Tris, pH 7.6. Sections were developed using a DAB reagent set (SeraCare, Milford, MA) and counterstained using hematoxylin (Sigma Aldrich, St. Louis, MO). Sections were dehydrated in graded ethanol solutions (70%, 90%, and 100%) and xylene prior to being coverslipped with Cytoseal (Thermo Fisher Scientific, Waltham, MA). Slides were digitally scanned using an Aperio Slide Scanner AT2 instrument (40X magnification; Aperio Technologies Inc., Vista, CA). Representative images of medullary asyn pathology were analyzed and captured with ImageScope software (Aperio Technologies Inc., Vista, CA). Medullar regions of interest were outlined and the percentage of DAB-positivity in each area was quantified using a modified Positive Pixel Count v9 algorithm within ImageScope.

Statistics

All statistics were performed in Graphpad Prism. ELISA data in Fig. 3 were assessed by paired t-test and repeated measures one-way ANOVA followed by Tukey's test, respectively. Survival curves were assessed with the Log-rank (Mantel-Cox) test with Holm-Šídák multiple comparison for pairwise comparison of all cohorts (Fig. 1b). One UB-312-treated animal and three adjuvant-treated animals were excluded from the recording of survival-free paralysis as they were euthanized due to fighting/penile prolapse in the absence of end-stage paralysis. Immunohistochemistry data (originally generated in Aperio ImageScope software) was analyzed using one-way ANOVA followed by Tukey's test (Fig. 2). Detailed descriptions of statistical tests and output can be found in supplemental table 1.

Results

To assess the effects of UB-312 in this seeded M83^{+/-} model, PFF-seeded M83^{+/-} mice were treated with (IM) injections of UB-312, adjuvant, or saline injections at 5 predetermined timepoints (Fig. 1a, Methods). Serum samples were taken ~ 24 hours prior to each injection (Fig. 1a, Methods). Mice were monitored until they displayed terminal bilateral hindlimb paralysis, at which point animals were sacrificed.

Survival curves using severe motor impairment as endpoint were generated for all cohorts (Fig. 1b). The survival of the UB-312-treated mice was significantly ($p = 0.0468$) prolonged as compared to that of the saline-treated mice with survival means of 124 and 110 days, respectively. The mean survival for adjuvant-treated mice was 115 days ($p = 0.2132$ for UB-312 treatment compared to adjuvant alone). Comparison of survival outcome between adjuvant-treated mice and saline-treated mice was similarly non-significant ($p = 0.2524$). Importantly, fight wounds and penile injuries necessitated that 3 male mice from the adjuvant-treated cohort and 1 male mouse from the UB-312 cohort were euthanized at 99, 104, 107, and 113 dpi, respectively. Resultantly, these mice were not included in the survival curves. It is possible that omission of 25% of adjuvant-treated animals has a confounding effect on interpretation of survival as it relates to the adjuvant group in particular.

To assess immunological response from UB-312 treatment, blood sera collected prior to each injection was tested against wildtype human α syn monomers and PFFs by ELISA. Initially, post-immune serum from weeks 3, 6, and 12 was diluted 1:100 and tested against wildtype human α syn monomers and PFFs by ELISA. Within the UB-312-treated group, no significant difference was detected for serum affinity to α syn monomers compared to affinity for PFFs (Fig. 3a-paired t-test $p = 0.0693$, correlation coefficient = 0.9339), so follow-up ELISA analyses were performed using only PFFs. Sera taken just before the 0 (pre-immune), 3, 6, and 12 week timepoints for UB-312 or saline treatment were tested across a dilution series (Fig. 1c). Pre-immune serum (serum collected at week 0) from saline or UB-312-treated controls did not produce an appreciable response to α syn PFFs. All post-immune serum from UB-312-treated animals produced a clear, dose-dependent response to α syn PFFs that was sustained at 12 weeks but not significantly different across weeks 3, 6, and 12 (Fig. 3b). This suggests that repeated UB-312-treatment of M83^{+/-} mice generated a robust anti- α syn response that persisted 6 weeks after the third treatment (12-week timepoint).

To evaluate neuropathological effects of UB-312, post-mortem histological assessment of CNS α syn pathology was performed in end stage tissue specimens (Fig. 2, Methods). Overt changes in the amount and distribution of α syn pathology were not observed across the three treatment cohorts, but quantitative assessments focused on the medulla region were also performed as it is a brain structure that reliably demonstrates robust pathology in this M83^{+/-} neuro-invasion model¹⁰. Two independent markers of α syn pathology, phospho-serine 129 (81A)¹² and carboxy (C)-terminal truncation at residue 103 (2G5)¹³ were used for this analysis. No significant difference was observed in the levels of α syn pathology in UB-312-treated mice as compared to saline or adjuvant controls (Fig. 2).

In summary, we observe an anti- α syn immune response and moderate bolstering of survival in the seeded M83^{+/-} mouse model upon peripheral treatment with UB-312, however UB-312 does not significantly modify CNS α syn pathology in this context.

Discussion

Mounting evidence suggests that bi-directional crosstalk between the gut and the CNS plays a role in Parkinson's and related diseases¹⁴, but a nuanced understanding of this role is still evolving. Synucleinopathy is detectable within the enteric nervous system at autopsy across nearly all PD cases¹⁵, and biopsy studies have shown α syn deposition in the stomach and intestines as much as 20 years ahead of PD diagnosis^{16–18}. While this peripheral α syn pathology is not generally associated with a significant neuronal loss within the enteric nervous system in PD¹⁹, it supports the ascending anatomic theory of gut-to-brain spread of pathology wherein α syn misfolding begins in the enteric nervous system and propagates to the brain via the vagus nerve. The M83^{+/-} CNS neuro-invasion model used here parallels this proposed periphery-to-CNS propagation in a rodent model^{10,20}. Here, we demonstrate an anti- α syn immune response and increase in survival in the seeded M83^{+/-} mouse model upon peripheral treatment with the α syn-targeting immunotherapeutic UB-312. Interestingly, this buffering of survival appears to occur in the absence of significant changes to CNS synucleinopathic burden, as assessed histologically. As brain tissue from this study was taken for whole-brain coronal sectioning, biochemical assessments of CNS α syn were not performed. Given that the development of synucleinopathy in these mice is a progressive process, it is also possible that increased survival of UB-312-treated animals versus saline-treated controls skews interpretation of CNS α syn burden insofar as longer-lived animals may tend to have a greater total pathological burden. As assessed, the moderate improvement in survival in the absence of significant CNS changes in pathology suggests the possibility that UB-312 treatment is affecting peripheral pools of α syn, but this study did not take peripheral tissues for immune profiling or pathological assessment that would be needed to better support this phenomenon.

UB-312 has previously demonstrated positive results in the Thy1SNC/15 mouse, which models a gradually progressive, early stage of synucleinopathy⁶. UB-312 testing in a phase I clinical trial also demonstrated a promising safety and target engagement profile in PD patients¹⁰. The work described here provides additional testing of UB-312 in assessment of efficacy in a relatively aggressive preclinical model of synucleinopathy, the seeded M83^{+/-} mouse model. Stimulation of anti- α syn immune activity paired with moderate improvement in survival suggests that UB-312 has an α syn targeting, salubrious effect in the context of this model. Future experimentation should include follow-up investigation of the possibility of a more peripheral treatment effect, as this would have important implications for UB-312 dosing considerations including route of administration, dose size, and intervention window.

Declarations

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Author Contribution Declaration

Conceptualization: JB, JD,BG; Methodology: KG,JB,JD,BG,MH; Investigation: KG,BB,JB,MH; Supervision: BG,MH; Writing-original draft: KG, MH; Writing-review and editing: All authors.

Competing Interests

At the time during which this work was conducted, Justin D. Boyd and Jean-Cosme Dodart were employees of Vaxxinity, Inc., a for-profit clinical-stage biotechnology company that developed UB-312.

Author Contribution

Conceptualization: JB, JD,BG; Methodology: KG,JB,JD,BG,MH; Investigation: KG,BB,JB,MH; Supervision: BG,MH; Writing-original draft: KG, MH; Writing-review and editing: All authors.

Data Availability

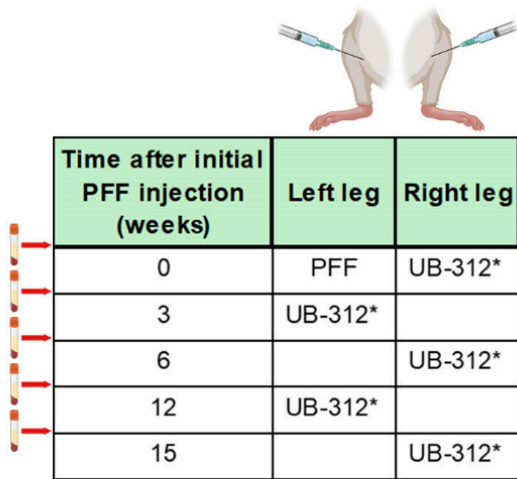
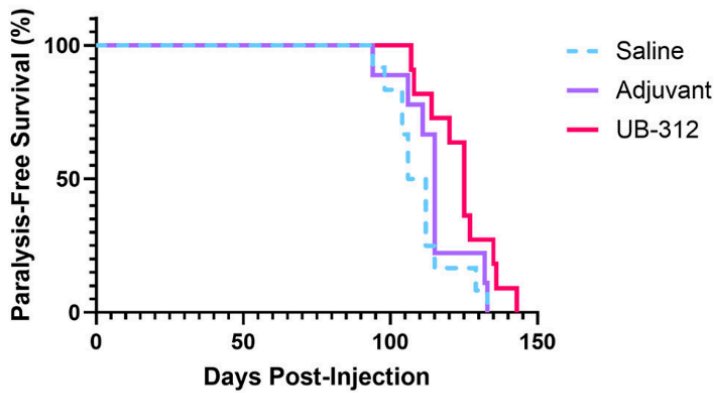
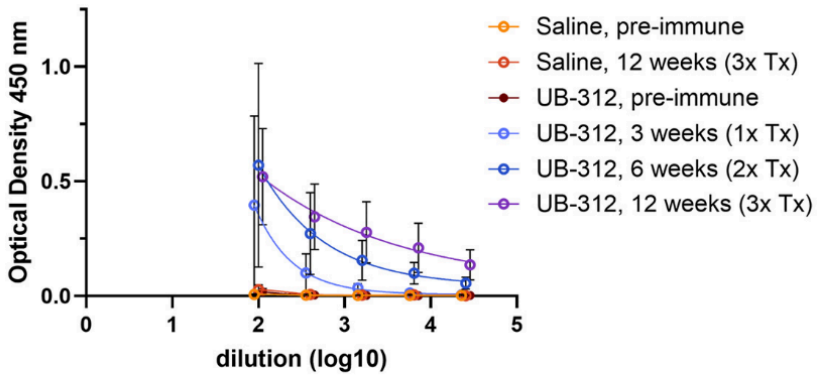
All original data discussed in this manuscript is represented in graphical form in primary figures. Excel-based raw data files for survival, serum ELISA, and histological quantitation can be provided by the corresponding author upon request.

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Figures

a**b****c****Figure 1**

Treatment with UB-312 delays terminal outcome in an M83^{+/-} α syn prion-type neuroinvasive model.

1a) Summary of experimental treatment schedule using M83^{+/-} mice. At 2 months of age (0 week timepoint), M83^{+/-} mice received separate, unilateral administrations of α syn PFFs and UB-312 in the opposite gastrocnemius muscle. 3, 6, 12, and 15 weeks later, animals received an intramuscular boost of

UB-312, with administrations alternating between left and right gastrocnemii. *Additional M83^{+/-} mouse cohorts received adjuvant or saline in place of UB-312 at all timepoints. Serum was collected from all animals by submandibular puncture 24 hours prior to each injection session.

1b) Paralysis-free survival of M83^{+/-} mice is prolonged with UB-312 treatment. Paralysis-free survival of all M83^{+/-} cohorts is presented in a survival curve. Days survived are reported from the initial PFF injection at 2 months of age (day 0). When assessed by log-rank (Mantel-Cox) test followed by Holm-Šídák multiple comparison, survival of UB-312-treated mice is significantly extended compared to that of the saline-treated mice ($p = 0.0468$). Note: Fight wounds and penile injuries necessitated that 3 male mice from the adjuvant-treated cohort and 1 male mouse from the UB-312 cohort were euthanized at 99, 104, 107, and 113 dpi, respectively. These mice were not included in the survival curves.

1c) UB-312 treatment induces elevated levels of anti-*asyn* antibodies in the serum. ELISA using *asyn* PFFs to coat plates. Pre-immune serum from saline or UB-312-treated animals shows negligible *asyn* PFF immune-reactivity. Meanwhile, serum taken after 1, 2, or 3 treatments with UB-312 (3, 6, and 12 weeks, respectively), demonstrates a pronounced, dose-dependent recognition of *asyn* PFFs compared to saline treatment. Dilution series is identical for all groups; clustered data points are staggered along the x-axis to enhance visual clarity.

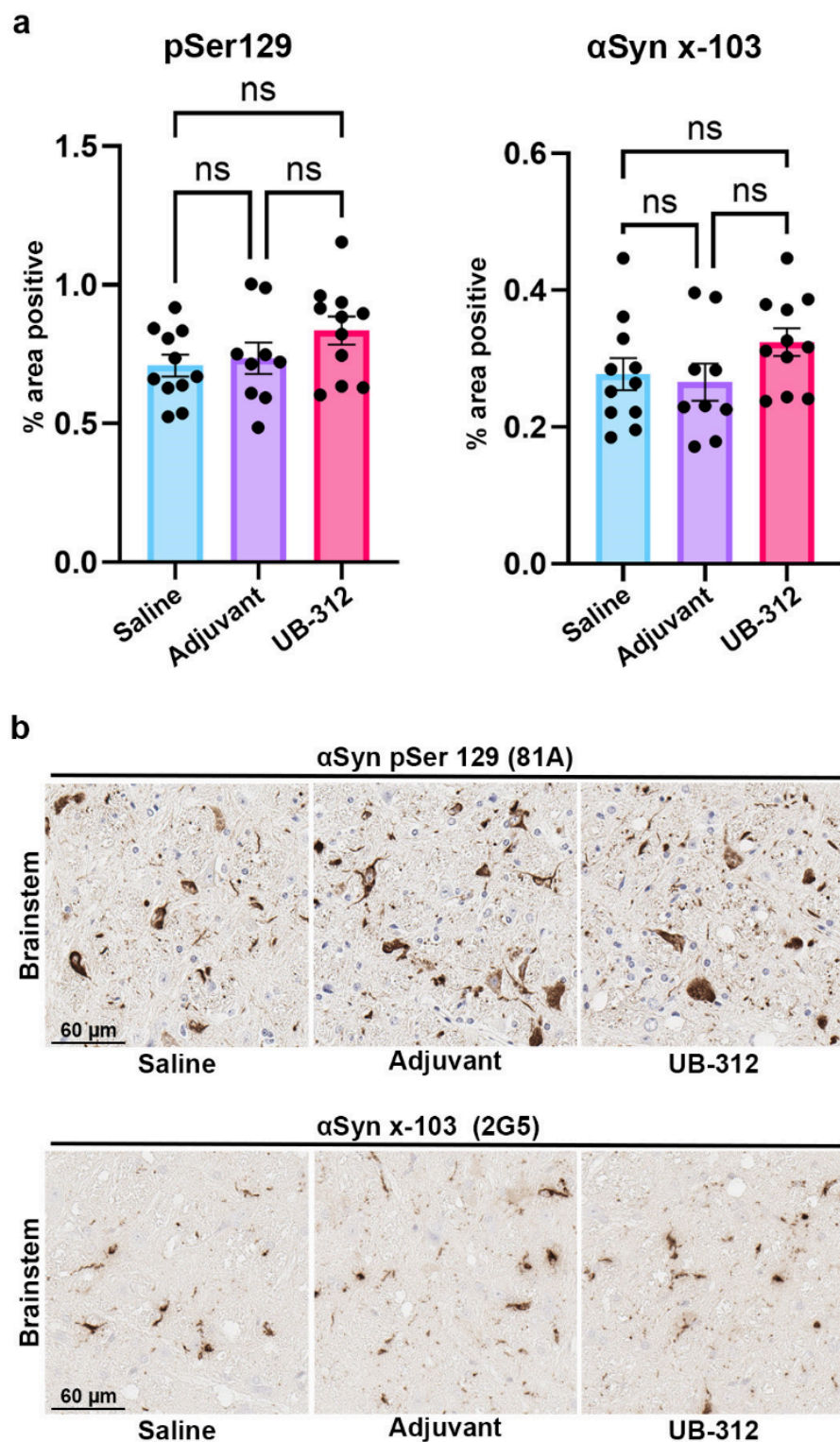


Figure 2

The levels of asyn CNS pathology was not altered by UB-312 treatment in terminal stage M83^{+/-} mice.

2a) Quantitation of medullar asyn pathology burden in treated M83^{+/-} mice. Medullar asyn pathology was assessed across all cohorts with anti-asyn antibodies targeting phosphorylation at serine 129 (pSer129, antibody 81A) or C-terminal truncation at residue 103 (αSyn x-103, antibody 2G5). In addition to the four treated animals excluded from the survival curve due to fight injuries (see Figure 1b), the

tissue from one saline-treated animal was not available for this analysis. For each stain, immuno-positive area is represented as a percentage of total area. No significant difference was observed between any treatment group when assessed by one-way ANOVA followed by multiple comparison using Tukey's test (n = 11, 9, 11). Data represented as mean +/- SEM. ns = not significant.

2b) Representative images of asyn pathology in the medulla of treated M83^{+/-} mice. Tissue sections were immunostained with antibodies 81A (pser129) or 2G5 (asyn x-103) and counter-stained with hematoxylin.

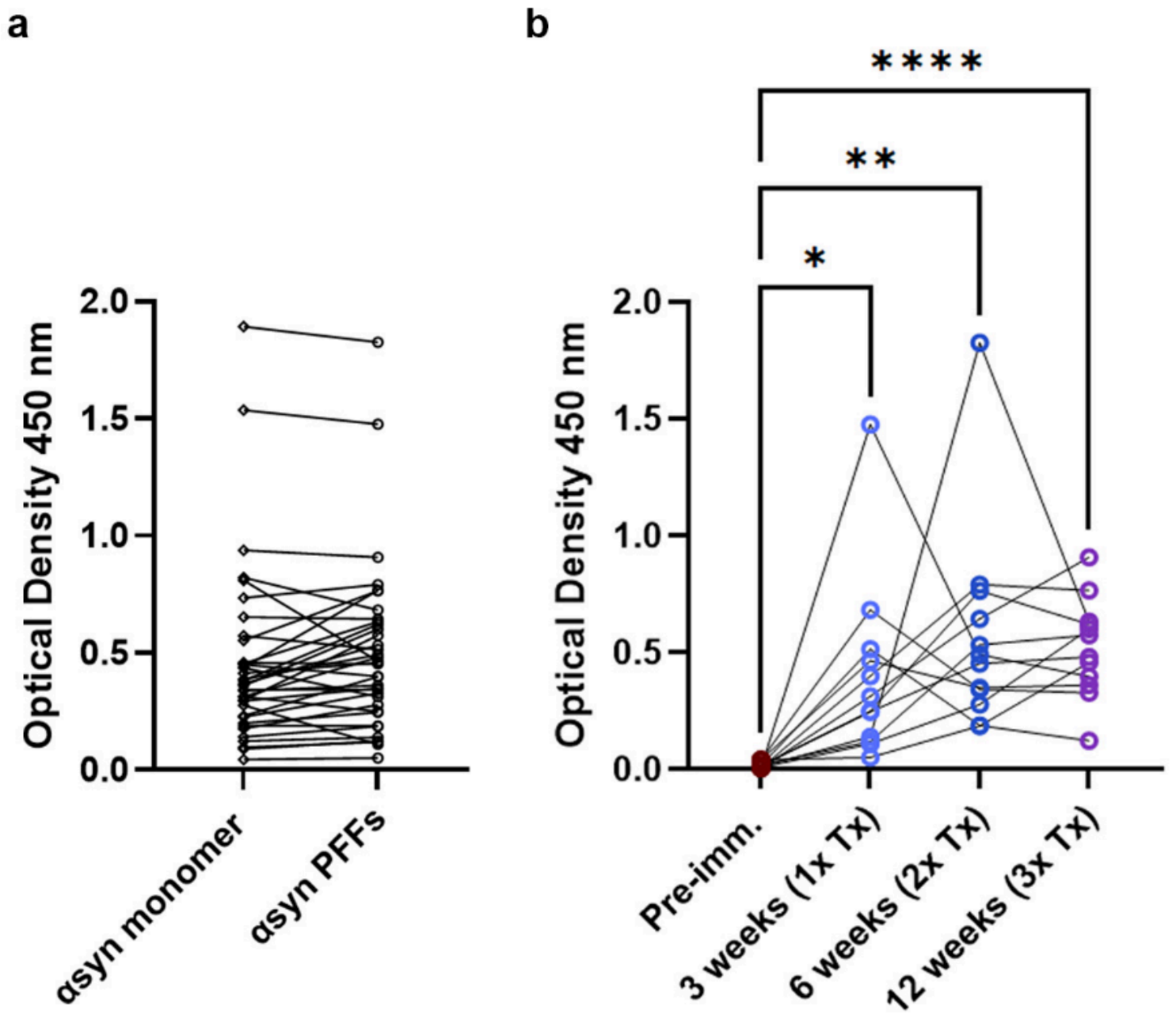


Figure 3

. Additional Serum ELISA data

a) Serum from UB-312-treated mice detects recombinant asyn monomers and PFFs by ELISA. Post-immune serum (taken at treatment weeks 3,6, and 12) from UB-312-treated animals was assessed for asyn monomer and PFF immunoreactivity at a 1:100 dilution. All post-immune samples from UB-312 treated animals were immunoreactive, and no significant difference was detected for reactivity to asyn monomers versus asyn PFFs (paired t-test $p=0.0693$, correlation coefficient $r=0.9339$). **b)** Longitudinal Immunodetection of asyn PFFs by serum of UB-312-treated mice. At a 1:100 dilution, serum from UB-312 treated-mice was widely immuno-positive at 3,6, and 12 week treatment timepoints. Post-immune serum immunopositivity was significantly elevated versus pre-immune samples from treatment week 0, but no significant difference in immunopositivity is seen across the 3,6, and 12 week timepoints.

* $p<0.05$; ** $p<0.01$; **** $p<0.0001$

Supplementary Files

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