# Safety and immunogenicity of the α-synuclein active immunotherapeutic PD01A in patients with Parkinson's disease: a randomised, single-blinded, phase 1 trial

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## Summary

**Background** **Robust evidence supports the role of α-synuclein pathology as a driver of neuronal dysfunction in Parkinson's disease. PD01A is a specific active immunotherapy with a short peptide formulation targeted against oligomeric α-synuclein. This phase 1 study assessed the safety and tolerability of the PD01A immunotherapeutic in patients with Parkinson's disease.**

**Methods** **We did a first-in-human, randomised, phase 1 study of immunisations with PD01A, followed by three consecutive study extensions. Patients aged 45–65 years with a clinical diagnosis of Parkinson's disease (≤4 years since diagnosis and Hoehn and Yahr Stage 1 to 2), imaging results (dopamine transporter single photon emission CT and MRI) consistent with their Parkinson's disease diagnosis, and on stable doses of Parkinson's disease medications for at least 3 months were recruited at a single private clinic in Vienna, Austria. Patients were randomly assigned (1:1), using a computer-generated sequence with varying block size, to receive four subcutaneous immunisations with either 15 μg or 75 μg PD01A injected into the upper arms and followed up initially for 52 weeks, followed by a further 39 weeks' follow-up. Patients were then randomly assigned (1:1) again to receive the first booster immunisation at 15 μg or 75 μg and were followed up for 24 weeks. All patients received a second booster immunisation of 75 μg and were followed up for an additional 52 weeks. Patients were masked to dose allocation. Primary (safety) analyses included all treated patients. These four studies were registered with EU Clinical Trials Register, EudraCT numbers 2011–002650–31, 2013–001774–20, 2014–002489–54, and 2015–004854–16.**

**Findings** **32 patients were recruited between Feb 14, 2012, and Feb 6, 2013, and 24 were deemed eligible and randomly assigned to receive four PD01A priming immunisations. One patient had a diagnosis change to multiple system atrophy and was withdrawn and two patients withdrew consent during the studies. 21 (87%) of 24 patients received all six immunisations and completed 221–259 weeks in-study (two patients in the 15 μg dose group and one patient in the 75 μg dose group discontinued). All patients experienced at least one adverse event, but most of them were considered unrelated to study treatment (except for transient local injection site reactions, which affected all but one patient). Serial MRI assessments also ruled out inflammatory processes. Systemic treatment-related adverse events were fatigue (n=4), headache (n=3), myalgia (n=3), muscle rigidity (n=2), and tremor (n=2). The geometric group mean titre of antibodies against the immunising peptide PD01 increased from 1:46 at baseline to 1:3580 at week 12 in the 15 μg dose group, and from 1:76 to 1:2462 at week 12 in the 75 μg dose group. Antibody titres returned to baseline over 2 years, but could be rapidly reactivated after booster immunisation from week 116 onwards, reaching geometric group mean titres up to 1:20218.**

**Interpretation** **Repeated administrations of PD01A were safe and well tolerated over an extended period. Specific active immunotherapy resulted in a substantial humoral immune response with target engagement. Phase 2 studies are needed to further assess the safety and efficacy of PD01A for the treatment of Parkinson's disease.**

**Funding** **AFFiRiS, Michael J Fox Foundation.**

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*Lancet Neurol* 2020; 19: 591–600

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## Introduction

α-Synuclein is a small, 14 kDa presynaptic protein that is essential for regulating synaptic activity and is involved in the compartmentalisation, storage, and recycling of neurotransmitters.[1] Robust evidence suggests that in patients with Parkinson's disease, dysregulated proteostasis of the α-synuclein protein leads to the formation of pathological α-synuclein species, including the build-up of oligomers and intracellular aggregates, which interfere with critical cell processes such as synaptic vesicular trafficking and mitochondrial and endolysosomal function.[2] There is also strong evidence for the occurrence of cell-to-cell transmission and prion-like spread of oligomeric α-synuclein assemblies.[3–5] Aggregated α-synuclein species are the primary structural component of Lewy bodies, which are a pathological hallmark of Parkinson's disease.[6] Several

**Research in context**

**Evidence before this study**
We searched PubMed and ClinicalTrials.gov for reports on immunotherapies for the treatment of Parkinson's disease or α-synuclein pathology, irrespective of the clinical stage of development, published in English or German, between database inception and Mar 26, 2020, using the search terms "α-synuclein", "vaccine", and "immunotherapy". We identified reports in a range of animal models that were published in the past two decades. Findings show that α-synuclein-specific monoclonal antibodies promote the degradation of α-synuclein aggregates, prevent cell-to-cell transmission, and reduce behavioural deficits. In two mouse models of Parkinson's disease, specific active immunotherapy with PD01A resulted in reduced levels of aggregated α-synuclein in the brain, and improved memory and motor defects. The search also yielded three phase 1 trials reporting acceptable safety and target engagement of passive immunotherapy with different monoclonal antibodies against α-synuclein in patient with Parkinson's disease. Five phase 1 or 2 trials using the passive immunisation approach in patients with Parkinson's disease are underway. There were no peer reviewed publications for specific active immunotherapy targeting α-synuclein, including the completed trial for the drug candidate PD03A in patients with Parkinson's disease, the results of which have not yet been published.

**Added value of this study**
To our knowledge, this is the first study of specific active immunotherapy against α-synuclein in patients with Parkinson's disease. Passive immunotherapy involves regular infusions of in vitro-produced monoclonal antibodies, whereas active immunotherapy involves eliciting a self-produced immune response, which is sustained over longer periods of time. In comparison to the three phase 1 passive immunotherapy trials, which have followed patients for up to 6 months, we show safety and sustained immunogenicity of the active approach over at least 3·5 years of follow-up.

**Implications of all the available evidence**
In this phase 1 study, immunisation with PD01A was safe when repeatedly administered and was well tolerated over an extended period. Immunisation resulted in a substantial humoral immune response against the target α-synuclein, and booster immunisations produced an effect that was higher than that seen after the initial priming immunisations. These data suggest immunisation with PD01A might be a promising strategy for long-term management of Parkinson's disease and justify moving forward with a phase 2 clinical programme.

transgenic animal models have shown that reducing oligomeric or aggregated forms of α-synuclein and reducing their cell-to-cell propagation might have disease-modifying effects.[7–10]

In the past decade, substantial progress has been made towards developing immunotherapeutic approaches targeting α-synuclein. For example, monoclonal α-synuclein-specific antibodies can promote the degradation of α-synuclein aggregates,[11] prevent cell-to-cell transmission,[12–14] and reduce behavioural deficits in animal models.[15] These findings have led to the development of several humanised or fully human monoclonal α-synuclein-specific antibodies, two of which have thus far shown acceptable safety,[16,17] and are currently being investigated.[18] Passive approaches targeting α-synuclein involve monthly, hospital-based, intravenous infusions of specific in vitro-produced monoclonal antibodies, whereas specific active immunotherapy uses immunisation to induce highly specific, self-produced, and sustained therapeutic antibody responses to prevent the spread of pathology, and potentially slow clinical progression with a much reduced treatment frequency.[18]

Applying this active immunisation approach to patients with Parkinson's disease, we have developed a novel immunotherapeutic (PD01A) involving immunisation with a short antigenic peptide, which mimics an epitope in the native C-terminal region of human α-synuclein.[19] The antigenic peptide (PD01), which consists of eight amino acids, is conjugated to the carrier protein keyhole limpet haemocyanin (KLH) and is absorbed to aluminium hydroxide leading to the drug product. PD01A is specifically designed to induce antibodies that selectively recognise α-synuclein aggregates with much lower affinity to monomeric forms and without reactivity to β-synuclein. The carrier protein provides the required T-helper epitopes for the induction of a long-lasting and boostable antibody response, whereas the antigenic component (ie, PD01) operates solely as a B-cell epitope and is responsible for the specificity of the humoral immune response. In two mouse models of Parkinson's disease, immunisation with PD01A resulted in reduced brain levels of aggregated α-synuclein and improved memory and motor defects.[19] Based on these preclinical studies, PD01A was advanced to clinical testing in a phase 1 clinical programme. This first-in-human study aimed to characterise the safety and tolerability of PD01A immunisation at two different doses in patients with Parkinson's disease.

## Methods

### Study design and participants

We did a patient-blinded, single-centre, randomised, parallel group, first-in-human study followed by three consecutive extension phases assessing two doses of PD01A (15 μg and 75 μg) at a single private clinic in Vienna, Austria. Doses correspond to the net antigen PD01 peptide amount of the applied drug product. The original study (substudy 1a) was a 52-week clinical trial

after which patients were observed for an additional 39 weeks of non-interventional follow-up (substudy 1b). Patients were subsequently invited to participate in two consecutive booster immunisation extension studies (substudy 2 [24 weeks] and substudy 3 [52 weeks]). The immunisation schedule (four primary immunisations plus two boosters) is shown in figure 1. Although all patients followed a similar flow through the studies, there were differences in duration of individual follow-up because of the need for patient reconsent and patient availability.

Both the non-interventional observational follow-up (substudy 1b) and the two booster studies (substudies 2 and 3) were not part of the original protocol and were designed and done after favourable safety and immunogenicity profiles had been demonstrated in substudy 1a. All studies were done in accordance with Good Clinical Practice, the Declaration of Helsinki with amendments (2013), Austrian Drug Law, and applicable international regulations. Each study was approved by the Ethikkommission Confraternität Privatklinik Josefstadt.

The inclusion and exclusion criteria are described in detail in the appendix (p 1). Briefly, patients aged 45–65 years with a clinical diagnosis of Parkinson's disease[20] (Hoehn and Yahr stages 1 to 2) given within the previous 4 years were eligible for inclusion. Patients had to have imaging results (dopamine transporter single photon emission CT [DAT-SPECT] and MRI) consistent with their Parkinson's disease diagnosis, and be on stable doses of Parkinson's disease medications for at least 3 months before study entry. During the study, adjustments to Parkinson's disease medications were made according to investigator judgement of clinical need. Patients were excluded if they had dementia, a history of autoimmune disease, recent (≤3 years) history of cancer, active infectious disease, relevant systemic illness (as judged by the investigator), a history of relevant psychiatric illness (eg, schizophrenia, bipolar disorder, or psychotic depression), or heredodegenerative disorders. Patients who had previously received experimental immunotherapeutic treatment or immunosuppressive drugs were also excluded. All patients provided written informed consent before enrolment.

## Randomisation and masking

Patients were randomly assigned in a 1:1 ratio to receive four immunisations with PD01A (15 μg or 75 μg) on an outpatient basis. The computer-generated randomisation code was provided by the study research organisation (Accelsiors Biostatistics, Budapest, Hungary), according to the random permuted blocks method with varying block size. Inclusion in substudy 1 was done in a stepwise manner. Initially, four patients (n=2 per dose group) were included. After their third immunisation, if no patient showed severe treatment emergent adverse events or a suspected unexpected serious adverse reaction, a further six patients (n=3 per dose group) could be

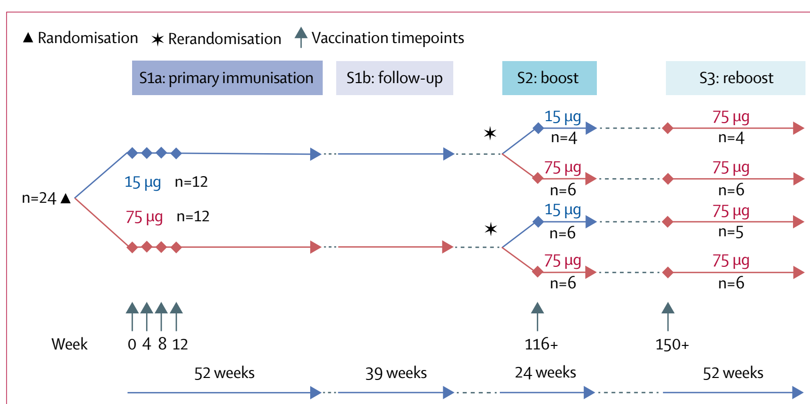


***Figure 1:*** **PD01A phase 1 study series schematic**

Schematic is not drawn to scale. Blood sampling occurred at screening and weeks 0, 2, 4, 8, 12, 26, 40, and 52 of S1a; at weeks 0, 13, 26 and 39 of S1b; at screening and weeks 0, 2, 4, 12, and 24 of S2; at screening and months 0, 2, 4, 6, 8, 10, and 12 of S3. S1a=substudy 1a. S1b=substudy 1b. S2=substudy 2. S3=substudy 3.

immunised. If no suspected unexpected serious adverse reactions developed until the third immunisation of the second step, the next patients could receive their first immunisation and recruitment could proceed without time-dependent recruitment conditions. Only patients were masked to dose allocation (immunisations were identical in appearance, both doses were prepared in 0·5 mL aluminium hydroxide-containing buffer). At the start of substudy 2, patients were rerandomised (1:1) to receive a booster immunisation of PD01A (15 μg or 75 μg). In substudy 3, all patients received a second booster of PD01A of 75 μg.

See Online for appendix

## Procedures

PD01A was manufactured by Bachem (Bubendorf, Switzerland) and the final formulation was produced by GL Pharma (Vienna, Austria). In substudy 1a, patients received one subcutaneous injection into the upper arm (alternating arms) of PD01A (15 μg or 75 μg) at baseline, and at weeks 4, 8, and 12. After rerandomisation in substudy 2, patients were given their first booster immunisation of PD01A (15 μg or 75 μg). The exact timing of the second booster injection of 75 μg in substudy 3 was flexible, depending on the level of immunisation-induced antibody response from the first booster immunisation in substudy 2.

After each of the six immunisations, patients were closely monitored for 1 h; blood pressure and heart rate were measured after 20 min and 60 min. Potential late-phase allergic reactions were assessed via a post-immunisation telephone interview the following day, and patients were instructed to carefully observe the injection site for 28 days after each immunisation. The severity of local reactions was rated by the investigator according to diameter of redness, swelling, and hardening at the subcutaneous injection site[21] (grading scheme for solicited local injection site reactions is provided in the appendix p 2). Patients also had a physical examination, including vital signs, standard haematology and

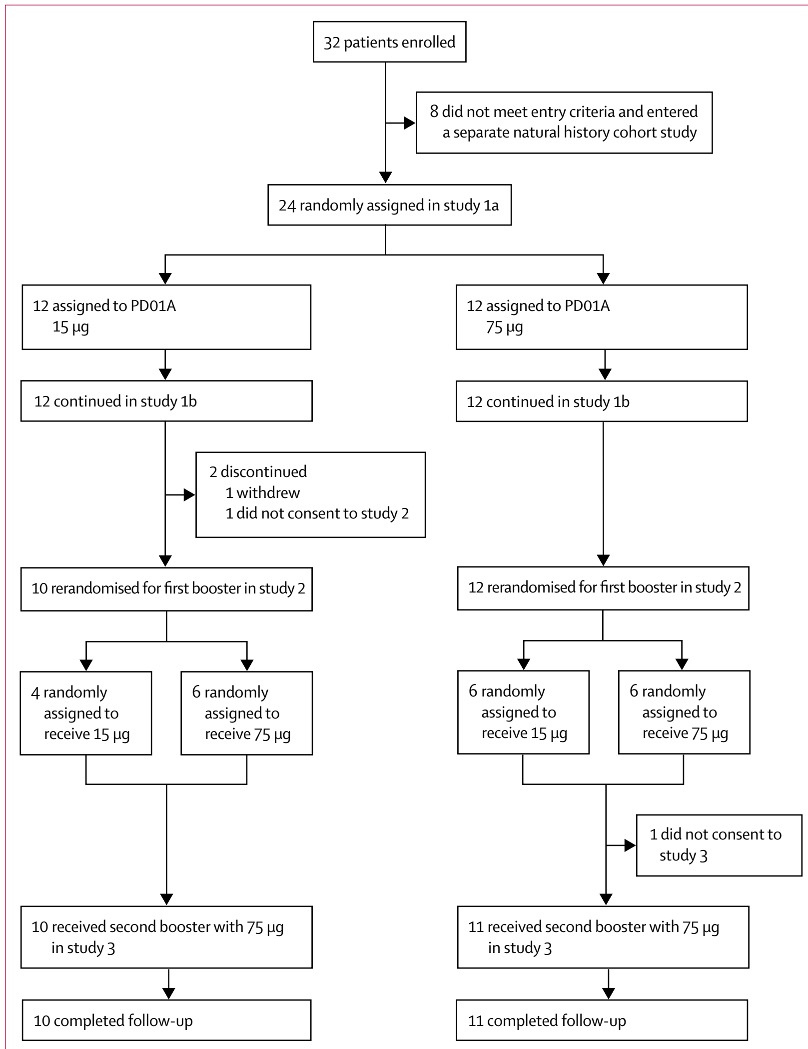


*Figure 2:* Trial profile

clinical chemistry assessments, urinalysis, electrocardiograms, serology, and hormones at each study visit. Patients completed a self-report safety diary throughout each study.

Additional safety assessments were done to complement the treatment-emergent adverse event reporting. Serial brain MRI with a 3.0 Tesla Philips Achieva Scanner (Philips Medical Systems, Best, Netherlands) with contrast to detect potential neuroinflammation and striatal DAT-SPECT scans (GE Healthcare, Chicago, IL, USA) using a Dual Head Nuclear Camera (Siemens, Erlangen, Germany) to assess disease pathology were done at baseline, and at weeks 26, 52, and 91. Additional MRI scans were also done at the time of the first booster immunisation and 24 weeks later (substudy 2).

Serum samples, collected during indicated visit time points (figure 1), were serially diluted (1:3 dilution steps) and titres were measured by an external provider (eBioscience, Vienna, Austria) using an ELISA validated to specifically detect IgG antibodies. CSF samples were collected at screening and at week 26 (substudy 1a). To check for a negative effect on disease progression, motor status was assessed at screening, weeks 26 and 52, and every 6 months thereafter using the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part 3 (in the ON state).[22]

A serial dilution of a human IgG pool coated to the ELISA plate was used as a calibration curve. End titres were defined as last serum dilution, which gave a signal that was higher than the signal of the calibration curve at penultimate dilution, and results are presented as geometric mean end titres.

Calibration-free antibody concentration analyses of sera (all patients) and CSF (isolated from three high-responding patients) were done using surface plasmon resonance (SPR) analysis (Biacore T200, Biacore, Uppsala, Sweden). For this analysis, biotinylated PD01 or control peptides were immobilised onto a precoated streptavidin chip. Selectivity of PD01A-induced antibodies for α-synuclein oligomers was also assessed using SPR; for this purpose, PD01A-induced antibodies isolated from plasma samples were immobilised to a CM5 chip using a human antibody capture kit (GE-Healthcare, Chicago, IL, USA). For binding analysis, a solution of either monomeric or oligomeric α-synuclein was applied to the sensor chip.

CSF samples, collected at screening and at week 26 (substudy 1a), were analysed for concentrations of total α-synuclein protein, amyloid β(1–42) protein, and phosphorylated tau protein using ELISA on material stored at –80°C. Since target engagement (ie, α-synuclein aggregates located in the brain) cannot be directly evaluated in this study, the concentration of oligomeric α-synuclein species in the CSF was analysed post hoc using ELISA as described by Majbour and colleagues;[23] the lower limit of this assay was reported to be 10 pg/mL. Briefly, the conformation-specific mouse monoclonal antibody Syn-O2 was used for capturing α-synuclein oligomers. Detection was done using the rabbit polyclonal antibody FL-140 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by the second step antibody goat anti-rabbit IgG labelled with HRP (Jackson ImmunoResearch, West Grove, PA, USA). Specific binding was visualised using an enhanced chemoluminescence substrate (SuperSignal ELISA Femto, Pierce Biotechnology, Rockford, IL, USA) and measured by an Envision plate reader (PerkinElmer, Waltham, MA, USA).

## Outcomes

The primary outcomes of safety and tolerability were assessed through the recording by the patient (diary data) and investigator (at each visit) of all local or systemic treatment-emergent adverse events, including serious adverse events or non-serious treatment-emergent adverse events, or both, possibly related to the study drug, and treatment-emergent adverse event-related study discontinuations. Any local adverse events of injection site pain,

itching, erythema, hyperthermia at injection site, oedema, induration, and granuloma recorded in a daily patient diary were always considered to be related to the immunisation. Patients also self-recorded any events of headache, myalgia, fever, fatigue, and nausea in their daily diaries. All other spontaneously reported treatment-emergent adverse events were also recorded by the patient in their diaries. We looked for any signs of an autoimmune response (eg, meningoencephalitis) based on clinical assessments, motor function, routine CSF analysis, and MRI.

The key secondary objective was to assess immunological activity after priming and booster immunisations. Antibody titres were determined for reactivity with PD01, the native epitope on the target α-synuclein protein, an irrelevant control peptide, and the carrier protein KLH (to confirm patients' immune competence). Furthermore, concentration determination of potential protein biomarkers, including total α-synuclein protein, Aβ(1–42) protein, and phosphorylated tau was done in the CSF at screening and week 26. Post-hoc analyses included CSF concentrations of α-synuclein oligomers at screening and week 26, PD01-specific serum antibody levels at week 12, and PD01-specific antibody levels in serum and CSF samples from three high-responding patients at week 26. Exploratory analyses evaluated the change from baseline in MDS-UPDRS part 3 scores.

| | PD01A 15 μg (n=12) | PD01A 75 μg (n=12) |
|---|---|---|
| Age, years | 55·4 (7·5) | 53·9 (7·3) |
| Sex | | |
| Male | 8 (67%) | 4 (33%) |
| Female | 4 (33%) | 8 (67%) |
| Weight, kg | 86·9 (12·2) | 75·0 (17·3) |
| Time since symptom onset, months | 31·4 (15·4) | 32·8 (16·6) |
| Hoehn and Yahr stage | | |
| 1·0 (unilateral disease) | 9 (75%) | 12 (100%) |
| 1·5 (unilateral disease plus axial involvement) | 3 (25%) | 0 |
| MDS-UPDRS scores | | |
| Part 1a | 0 | 0 |
| Part 2 | 5·8 (1·0–16·0) | 4·2 (0·0–11·0) |
| Part 3 | 11·9 (2·0–24·0) | 12·3 (2·0–26·0) |
| Levodopa use | 9 (75%) | 7 (58%) |
| Months on treatment | 13·6 (2·0–38·0) | 11·7 (1·0–39·0) |
| Dopamine agonist use | 9 (75%) | 9 (75%) |
| Months on treatment | 20·9 (1·0–57·0) | 17·2 (2·0–59·0) |
| MAO-B inhibitor use | 2 (17%) | 1 (8%) |
| Months on treatment | 17·5 (6·0–29·0) | 6·0 (6·0–6·0) |
| Levodopa dose equivalent | 432 (264) | 344 (248) |

Data are mean (SD), n (%) , or mean (range). MDS-UPDRS=Movement Disorder Society Unified Parkinson's Disease Rating Scale. MAO-B=monoamine oxidase B.

***Table 1:*** **Baseline characteristics of all randomly assigned patients**

## Statistical analysis

The study was designed to have an accuracy of estimation for frequent adverse events and side-effects (35% occurrence) of plus or minus 30% for ten patients and plus or minus 20% for 20 patients (two-sided CIs). To achieve ten completers in each group, 24 patients were treated with the study drug, assuming a dropout rate of 20% (up to week 26 in substudy 1a) in each treatment group.

All four studies (ie, four follow-up periods of the same patients, collected in separate substudies) were integrated to provide an overall analysis. Analyses were done on the integrated safety set, which included all patients who entered into substudy 1a and received at least one immunisation. Patients were analysed according to their sequence of treatment. For the safety analyses, pooled treatment groups (pooled 15 μg and 75 μg) were presented based on the study dose given in substudy 1a, irrespective of their first booster dose in substudy 2. Data are primarily descriptive, with no imputation for missing data. Immune response parameters are also presented according to the sequence of treatment (eg, 15 μg in substudy 1a and 75 μg in substudy 2 is referred to as 15 μg, 75 μg).

Immune responders (seroconversion) were defined as PD01A-immunised patients with a PD01 titre ratio at least four times baseline value. Immune response rates were compared across groups by counting the number of individuals above the respective cutoff. To evaluate dose dependency of the booster application, an additional analysis was done by grouping the patients based on the first booster dose they received (ie, 15 μg or 75 μg).

Exploratory analyses of change from baseline in MDS-UPDRS part 3 scores were done using a mixed model with repeated measures with terms for age, treatment group, visit (categorical 6 months or 12 months), and treatment by visit interaction and baseline of the outcome as covariates. The covariance structure used was variance components. All exploratory comparisons were hypothesis generating and done at the two-sided 5% level, with no corrections for multiple comparisons. In addition, post-hoc analyses tested for an association between baseline MDS-UPDRS part 3 scores and oligo-α-synuclein concentration in the CSF at baseline based on Pearson's correlation coefficient. Levodopa dose equivalent to the conventional Parkinson's disease medications were estimated using the calculation recommended by Tomlinson and colleagues.[24]

Statistical analyses were done using SAS, version 9.3. Studies 1a, 1b, 2, and 3, were registered at EudraCT (2011–002650–31, 2013–001774–20, 2014–002489–54, and 2015–004854–16, respectively).

## Role of the funding source

Authors employed by AFFiRiS were involved in the study design, data collection, data analysis, data interpretation, and in the writing of the report. All authors who are AFFiRiS employees, the first author, and the statistician

| | 15 µg PD01A (n=12) | 75 µg PD01A (n=12) |
|---|---|---|
| Overall adverse event frequency | 12 (100%) | 12 (100%) |
| Treatment-related adverse events | 11 (92%) | 11 (100%) |
| Treatment-related severe adverse event | 5 (42%) | 3 (25%) |
| Serious adverse event | 5 (42%) | 2 (17%) |
| Treatment-related serious adverse event | 0 | 0 |
| Adverse event leading to withdrawal | 0 | 0 |

Data are n (%). Pooled data are presented for the full follow-up (ie, across all substudies). Pooled treatment groups (pooled 15 µg and 75 µg) are presented based on the study dose given in substudy 1a, irrespective of their first booster dose in substudy 2.

*Table 2:* **Adverse events**

(CS) had full access to all data in the study. The corresponding author had final responsibility for the decision to submit for publication.

## Results

The clinical programme was conducted from Feb 14, 2012, through Feb 18, 2017. Between Feb 14, 2012, and Feb 6, 2013, 32 patients with early idiopathic Parkinson's disease were enrolled into substudy 1a. Of these patients, 24 (75%) met inclusion criteria and were randomly assigned to receive at least four immunisations of PD01A at 15 µg or 75 µg in substudy 1a. One patient had a diagnosis change to multiple system atrophy during substudy 1b and was withdrawn, one patient did not consent to enter substudy 2, and one did not consent to enter substudy 3; thus, 21 (87%) patients had all six immunisations and completed a range of 221 to 259 weeks of follow-up (figure 2). Because of individual differences in the time between substudy 1 and substudy 2, the first booster was given 25·2–35·2 months after the first primary immunisation (substudy 1a). The second booster injection took place when the patient's immunisation-induced antibody response was close to baseline, at between months 38·9 and 47·5 (median 43·4; 21 patients).

Patients' demographics and baseline characteristics are presented in table 1. All patients were white and the overall (all groups combined) mean age was 54·9 years (SD 6·74; range 43–65). Baseline characteristics were similar across the two groups, with the exception of the duration of levodopa and monoamine oxidase B (MAO-B) inhibitor use, which was longer in those receiving the 15 µg dose of PD01A compared with the 75 µg dose group. Patients received levodopa, pramipexole, rasagiline, ropinirole, and rotigotine at baseline.

Across the follow-up period, a total of 500 treatment-emergent adverse events involving all patients were recorded. The overall incidence of treatment-emergent adverse events are given in table 2, and further details of treatment emergent adverse events broken down by study periods are given in the appendix (p 3). With the exception of expected local injection site reactions, most adverse events were considered unrelated to study treatment. Across the two treatment groups, the only systemic treatment-emergent adverse events considered to be potentially treatment related and reported in at least two patients were fatigue (n=4), headache (n=3), myalgia (n=3), muscle rigidity (n=2), and tremor (n=2). The boost immunisations in substudies 2 and 3 had little effect on the safety profile of either dose compared with the initial immunisation in substudy 1a, except sporadic and transient elevations of liver enzymes (n=1), an increased incidence of infections including conjunctivitis and nasopharyngitis (n=3), and sporadic metabolism and nutrition disorders (n=2). No patient discontinued because of treatment-emergent adverse events and there were no deaths.

Transient local injection site reactions were common, affecting all but one patient. Site reactions were seen (for 15 µg and 75 µg dose groups) in eight (67%) of 12 patients in the 15 µg group and nine (75%) of 12 patients in the 75 µg group during substudy 1; seven (70%) of ten patients in the 15 µg group and 11 (92%) of 12 patients in the 75 µg group at the first booster immunisation in substudy 2; and seven (70%) of ten patients in the 15 µg group and ten (91%) of 11 patients at the second booster immunistion in substudy 3. Across both treatment groups, the most common local injection site reaction was erythema, which accounted for 52 (22%) of 238 reported injection site reactions; the only severe site reactions were reported at the second booster immunisation (12 events reported in eight patients). All injection site reactions throughout the study were transient and resolved within a mean of 4 days (range 1–9). Across both treatment groups, seven patients had at least one serious adverse event, none of which was judged as related to study treatment (serious events of back pain, meniscus injury, cervical dysplasia, cholelithiasis, femur fracture, and arthralgia were each reported in one patient; another patient had an increase in liver enzymes, aortic aneurysm, coronary artery disease, and erysipelas). Pooled analyses of the 15 µg and 75 µg doses showed some increases in blood pressure compared with baseline (n=4 with 15 µg, and n=3 with 75 µg).

No MRI abnormalities emerged post baseline, with the exception of one microhaemorrhage in the 15 µg group, which was not considered related to the study drug. None of the patients developed meningoencephalitis and none of the immunisations resulted in a clinically detectable type 1 allergic reaction. DAT-SPECT examinations did not show statistically significant changes up to 91 weeks in study 1 (appendix p 4). MDS-UPDRS part 3 scores were generally stable across the studies. Mean MDS-UPDRS part 3 scores in the pooled 15 µg group were 11·9 (SD 8·2) at baseline and 12·5 (14·8) at the last visit. In the pooled 75 µg group, MDS-UPDRS part 3 scores were 12·3 (7·2) at baseline and 8·6 (7·7) at the last visit. Levodopa dose

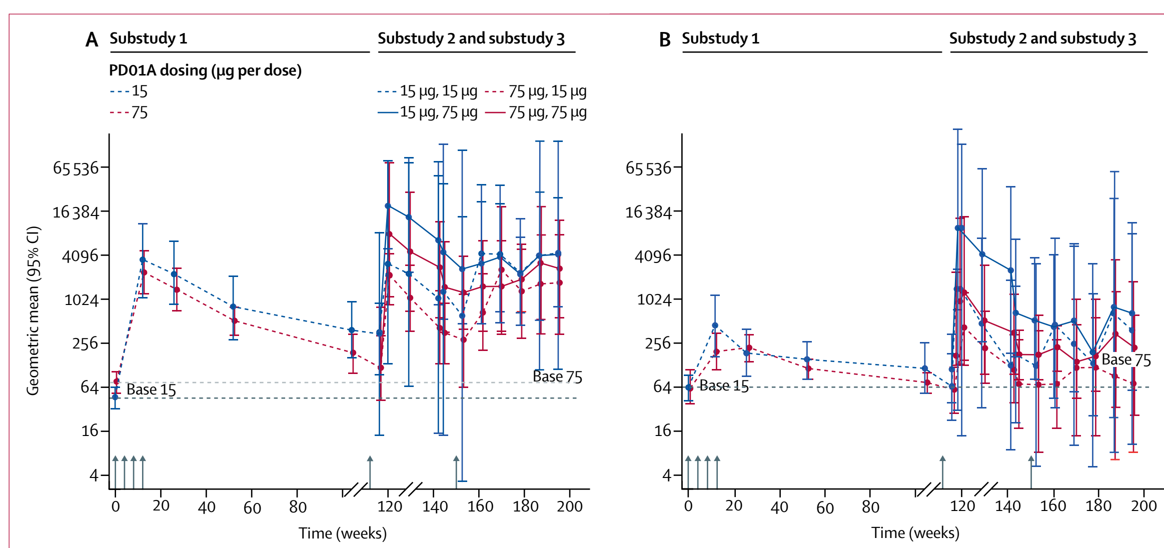


*Figure 3:* **Geometric mean antibody titres over time for PD01 peptide (A) and for native target sequence on α-synuclein (B)**
Time refers to the study week, not accounting for varying gaps between studies. Arrows reflect the timepoint of the immunisation. The exact timing of the second booster injection of 75 µg in substudy 3 was flexible, depending on the level of immunisation-induced antibody response from the first booster immunisation in substudy 2. In substudy 1, cohorts were either immunised with 15 µg or 75 µg. In substudy 2 patients were rerandomised (1:1) to receive either 15 µg or 75 µg booster, resulting in four groups (15 µg in substudy 1 and 15 µg in substudy 2; 15 µg in substudy 1 and 75 µg in substudy 2; 75 µg in substudy 1 and 15 µg in substudy 2; and 75 µg in substudy 1 and 75 µg in substudy 2)

equivalents increased from 355 mg at baseline to 587 mg at the final visit.

No significant changes were reported for laboratory parameters, except for sporadic and transient elevations of liver enzymes known to be associated with standard treatments of Parkinson's disease (appendix p 4). Mean erythrocyte counts, haemoglobin, bilirubin, or lactate dehydrogenase did not significantly change over time.

A substantial IgG antibody response against PD01 was observed after three priming immunisations with a maximum titre being achieved at week 12 and slowly declining back to baseline thereafter (half-life of 10–12 weeks). The geometric group mean titre of antibodies against the immunising peptide PD01 increased from 1:46 at baseline to 1:3580 at week 12 in the 15 µg dose group, and from 1:76 to 1:2462 at week 12 in the 75 µg dose group (figure 3). Post-hoc analyses showed that these three priming immunisations elicited a PD01-specific humoral immune response in ten (91%) of 11 patients in the 15 µg group, and all patients (12 [100%] of 12) in the 75 µg group. Thus, at week 12, most patients met the predefined cutoff for seroconversion against PD01 at a factor level of at least four times baseline.

To collect information about α-synuclein reactivity of PD01A-induced antibodies, the immune response against the corresponding native target sequence of α-synuclein was also determined. Results demonstrated good cross-reactivity against the native target sequence with lower geometric mean titres. Titres increased from 1:67 at baseline to 1:330 after three immunisations in the 15 µg group, and from 1:68 to 1:171 in the 75 µg group (figure 3). Importantly, the IgG response against the α-synuclein epitope displayed a time profile similar to that observed with the PD01 peptide. In addition, all patients developed an immune response to the carrier protein KLH with a time profile similar to that observed with the immunising PD01 peptide and target α-synuclein protein (appendix p 5).

The first booster immunisation in substudy 2 induced higher PD01 peptide titres than those observed with the four priming immunisations in substudy 1a with peak titres achieved 2 weeks after administration. The geometric group mean titres obtained for these four groups (ie, 15 µg, 15 µg; 15 µg, 75 µg; 75 µg, 15 µg; 75 µg, 75 µg), were 1:7386, 1:20218, 1:3891, and 1:9720, respectively (figure 3). Geometric group mean titres to the native epitope on a-synuclein also increased to 1:1403, 1:4209, 1:389, and 1:810, respectively, after the booster application (figure 3). Post-hoc analyses of the booster doses revealed that the 75 µg dose elicited significantly higher titres than the 15 µg dose against both PD01 peptides (p=0·023; appendix p 5). Thus, the primary immunisation in substudy 1 produced a substantial memory effect that was reactivated and augmented during the booster immunisations. In substudy 3, administration of the second booster (ie, 75 µg) resulted in a persistence of the humoral immune response until study end (figure 3).

PD01-specific antibody concentration in serum samples derived from week 12 (SPR analysis substudy 1) ranged from 0·5 µg/mL to 18·0 µg/mL, reaching a mean serum antibody concentration of 5·4 µg/mL. After booster application in substudy 2, serum antibody concentrations increased significantly compared with week 12 (p=0·031), reaching between 1·0 µg/mL and 114·0 µg/mL, with a

mean antibody concentration of 19·6 μg/mL. Serum antibody titre and antibody concentration were significantly ($p<0·0001$) and strongly correlated (Pearson correlation coefficient across all samples $r=0·87$). SPR analysis of week 26 CSF and serum samples from three high-responding patients revealed that the concentration of PD01A elicited antibodies in CSF relative to serum was about 0·3%.

Independent of the dose group, the concentration of total α-synuclein in CSF did not change after immunisation. However, post-hoc analysis of week 26 samples revealed a mean reduction of 51% in CSF oligomeric α-synuclein in patients immunised with the 75 μg dose. Treatment with the 15 μg dose also led to a slight decrease in the oligomeric α-synuclein (appendix p 5). Further analyses revealed a significant correlation (Pearson correlation coefficient $r=0·515$ and $p=0·020$) between the oligo-α-synuclein concentration in the CSF at baseline and baseline MDS-UPDRS part 3 scores. The concentrations of amyloid β, total tau protein, and phosphorylated tau protein (181) did not change over time after immunisation (appendix p 4). In addition, high selectivity of PD01A-induced antibodies (isolated from three patient sera) towards oligomeric α-synuclein structures was shown in a SPR-based assay (appendix p 6).

## Discussion

The results of this phase 1 study suggest that immunisation with PD01A is safe when repeatedly administered, and is well tolerated over an extended period. Immunisation resulted in a substantial increase in titres against the immunising peptide PD01 at week 12 (day of fourth priming immunisation), which translated into a humoral immune response against the α-synuclein target epitope, approximately one order of magnitude lower. Booster immunisations produced an immediate effect on all analysed titres that was substantially above those observed after the priming immunisations. The immunisation had a favourable safety profile, with transient local injection-site reactions being the main treatment-emergent adverse events, consistent with other vaccination therapies,[25] and did not lead to study withdrawal.

To ensure accuracy of reporting, patients kept detailed safety diaries in each study. Most systemic treatment-emergent adverse events were attributed to underlying disease progression or concomitant symptomatic therapies. There were no signs of dose-dependent safety patterns when comparing the high-dose treatment with that of the 15 μg dose group despite a five times higher dose. Likewise, there was no suggestion of cumulative toxicity over time or with booster injections. Of note, the boost immunisations had little effect on the adverse event profile. The active immunisation did not appear to cause any worsening in clinical status as shown by the MDS-UPDRS part 3 scores as well as the DAT-SPECT and MRI results over the extended study period. This is in contrast to previously published data which reports a worsening in MDS-UPDRS part 3 scores in a similar population of patients with early Parkinson's disease.[26]

Active immunisation with PD01A led to the induction of a specific immune response with high selectivity for higher order α-synuclein structures. A substantial increase in humoral response observed after booster immunisation in substudy 2, almost 2 years after the first immunisation, suggests that the priming immunisations produced a substantial memory effect for the immunising peptide PD01 and the α-synuclein target epitope. The observation of lower titres for the α-synuclein target epitope compared with the immunising peptide can partly be explained by the binding of product-induced antibodies to the target structure (ie, masking the number of detected antibodies). We tested PD01A at 15 μg and 75 μg. Although no clear dose response was seen in immune response parameters after the primary immunisation schedule, the 75 μg dose resulted in fewer interindividual variations in target-specific antibody response. Independent of the priming immunisation dose, the 75 μg booster dose was associated with a more homogeneous and significantly higher immune response than seen with the 15 μg booster, thus addressing the possible issue of immunosenescence. The 75 μg dose was therefore exclusively used as the second booster dose in substudy 3. Specific serum antibody concentrations (mean of 19·6 μg/mL) were lower than reported for monoclonal antibody studies, which have peak levels of more than 1000 μg/mL immediately after antibody infusion for the highest dose studied.[16,17] However, the antibody concentration dynamics might be substantially different depending on whether they result from active or passive immunotherapy and no data on the concentration of antibodies required to achieve a therapeutic clinical effect is as yet available.

Accumulating evidence suggest that soluble α-synuclein oligomers are a key pathogenic factor in Parkinson's disease and that reducing the levels of α-synuclein oligomers could potentially have a disease-modifying benefit in patients with Parkinson's disease.[18] In SPR analyses, PD01A-induced antibodies were found to bind α-synuclein oligomers with high selectivity. Using highly specific and sensitive ELISA assays, we found that active specific immunisation with PD01A was associated with substantial reductions in CSF levels of α-synuclein oligomers, which can be interpreted as a sign of in vivo target engagement (and no changes in total α-synuclein, or amyloid β[1–42] protein and phosphorylated tau). Moreover, despite a small sample size, oligomeric α-synuclein levels correlated with MDS-UPDRS part 3 scores at baseline. To our knowledge, this is the first report of an active anti-α-synuclein immunotherapy associated with reduction of CSF α-synuclein oligomers in patients with Parkinson's disease and points to the possible development of a useful biomarker for staging of Parkinson's disease pathology.

There are several limitations to this first-in-human clinical programme. Although the integrated study

database provides a continuous follow-up of most patients, studies 1b–3 were not preplanned. Our substudies of target engagement evolved in response to developing technologies and were thus done post hoc. When the study started, the majority of the exploratory biomarker assessments were in a planning status and it was unclear whether it would be possible to develop relevant assays. Other key limitations include the absence of a placebo group and the small sample size. The cohorts were from a single centre, were demographically similar, diagnosed within the past 4 years, and were all on stable Parkinson's disease medication. Finally, although we assessed MDS-UPDRS part 3 scores, the primary purpose was to assess safety. The open nature of this study, with multiple adjustments of symptomatic medications, precludes any conclusions on potential disease-modifying efficacy. Although there have been reports of MAO-B inhibitors acting as immunostimulants,[27] this has not yet been confirmed. It is considered unlikely that the symptomatic medications influenced the immune response to therapy. Although the correlations between CSF α-synuclein oligomers and MDS-UPDRS part 3 scores are intriguing, the clinical efficacy of the immunotherapeutic requires further exploration.

In summary, the safety profile and positive antibody response of PD01A supports the further development of this immunotherapeutic for the treatment of Parkinson's disease in a phase 2 clinical trial. Current immunotherapeutic strategies to combat neurodegenerative disorders comprise passive immunotherapy (eg, regular injections of monoclonal antibodies against specific endogenous targets) and specific active immunotherapies (eg, vaccines). Targeting α-synuclein in patients with Parkinson's disease using antibody-based approaches is considered a promising strategy, because pathological and genetic evidence strongly implicates toxic effects of aggregated α-synuclein in the pathophysiology of Parkinson's disease. Although monoclonal antibodies are becoming a mainstay for many noncommunicable disorders such as cancer,[28] they face functional limitations because of their relatively short in vivo half-lives, necessitating frequent administration, which is associated with high treatment costs. Specific active immunotherapy approaches could circumvent these drawbacks by stimulating a self-produced, long-lasting immune reaction. Our findings of a sustained α-synuclein-specific antibody response, suggests immunisation with PD01A might be a promising strategy for long-term management of Parkinson's disease.

#### Contributors

AS designed and oversaw the execution of all studies. DV and CT-H led the patient testing and data collection. AK oversaw the execution of substudy 3 and the planning and implementation of the integrated data base for studies 1–3. WP, PL, GS, and RM wrote the first draft of the Article. DW, EM, AM, and GG did immunogenicity analyses. NM, NV, and OE-A did the oligomeric α-synuclein analyses. CS had statistical oversight. All authors contributed to the writing and revision of the Article.

#### Declaration of interests

DV and CT-H were investigators and received funding from AFFiRiS. WP reports receiving personal fees from AbbVie, AFFiRiS, AstraZeneca, BIAL, Boston Scientific, Britannia, Intec, Ipsen, Lundbeck, NeuroDerm, Neurocrine, Denali Pharmaceuticals, Novartis, Orion Pharma, Prexton, Teva, UCB Pharma, and Zambon; receives royalties from Thieme, Wiley Blackwell, Oxford University Press, and Cambridge University Press; and grant support from the Michael J Fox Foundation, European Commission (EU FP7), and Horizon 2020. AK, PL, DW, EM, GS, and RM are employed by AFFiRiS. GG, AS, and AM were employed by AFFiRiS at the time of study. NM, NV, and OE-A did the oligomeric α-synuclein analyses funded by AFFiRiS. CS did the statistical analysis funded by AFFiRiS.

#### Data sharing

Deidentified data and study protocols used in this publication will be made available to qualified researchers who provide a valid research question. Please direct inquiries to the corresponding author.

#### Acknowledgments

The study was funded by AFFiRiS, with support from the Michael J Fox Foundation, Clinical Intervention Award 2011 (an Edmond J Safra Core Program for postdoctoral research). We thank the AFF008 DSMB board for their feedback on the study (appendix p 1). We thank the Michael J Fox Foundation, especially Todd Sherer and Kuldip Dave for their continued support. Medical writing support was provided by Anita Chadha-Patel (ACP Clinical Communications, funded by AFFiRiS).

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