ACI-7104.056, AN ACTIVE IMMUNOTHERAPY FOR SYNUCLEINOPATHIES, INDUCES A STRONG AND SUSTAINED ANTIBODY RESPONSE AGAINST ALPHA SYNUCLEIN IN NON-HUMAN PRIMATES.

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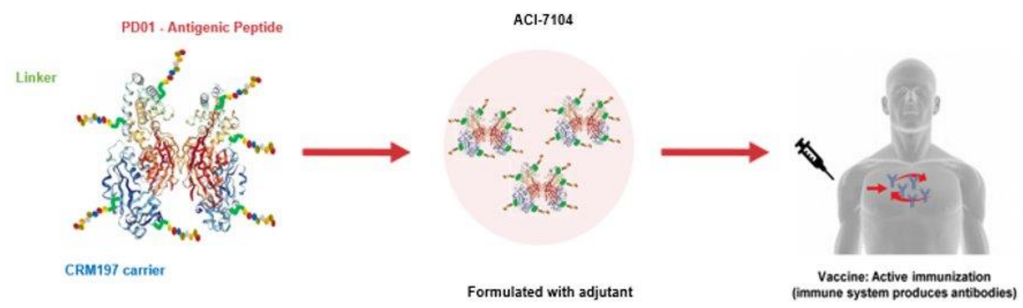
Guenther Staffler, Rakel Carpintero, Emma Fiorini, Stefanie Siegert, Maelle Verardo, Nathalie Chuard, Aurélie Guillaume-Gentil, Lorène Aeschbach, Nadine Ait-Bouziad, Ilonka Van Hoof, Francesca Capotosti, Elpida Tsika, Marija Vukicevic, Andrea Pfeifer, Marie Kosco-Vilbois.
AC Immune SA - Lausanne (Switzerland)

Objective

The accumulation of misfolded alpha synuclein (a-syn) and the cell-to-cell transmission of resulting a-syn aggregates propagate disease pathology in Parkinson's disease (PD) and other synucleinopathies. Thus, a-syn is considered a target for disease modification. ACI-7104.056, an a-syn-targeting immunotherapy currently being tested in a phase 2 clinical trial (NCT06015841), is designed to trigger an antibody response specifically binding to a-syn aggregates to halt their propagation. Here, we have assessed the safety and immunogenicity of ACI-7104.056 in non-human primates (NHP) and further characterized the quality of induced antibodies.



1. **Vaccine containing a-syn derived peptide (PD01)** (designed to raise antibodies against pathological a-syn species)
2. **High and boostable immunogenicity against a-syn** (CRM197: NON-a-syn T-helper activation)
3. **Well established adjuvant enhances overall immune response**

***Figure 1**: ACI-7104.056 is composed of three main subunits*

Methods

NHPs received multiple intramuscular injections of ACI-7104.056 and serum was collected at different time points.

Anti-aggregate a-syn IgG titer determination: Antibody levels were determined in serum samples of all animals using MSD 96-well standard microplates precoated with HNE-stabilized a-syn aggregates. Titres were calculated from the standard curve using a four-parameter logistic fitting with $1/y^2$ weighting using the MSD software and expressed in arbitrary unit/mL (AU/mL).

Competitive inhibition experiments: In competitive inhibition experiments, sera from NHPs were pre-incubated with increasing concentrations of a-syn monomers or a-syn aggregates in solution before being analyzed in an ELISA plate coated with a-syn aggregates. Inhibition was quantified as reduction of the signal as compared to the untreated sample (Figure 2A & Figure 4).

Neuronal seeding assay: Rat primary neurons were plated in 96-well plates. NHP sera were pre-incubated with human pre-formed fibrils (hPFF) and subsequently added to the neuronal cultures and incubated for eight days. Cells were fixed and stained for Microtubule-associated protein 2 (MAP2) and alpha synuclein pS129 and imaged (Figure 2B & 6).

Immunohistochemical analysis (IHC): Frozen human brain sections from a PDD(1) donor and from a MSA(2) donor were used for IHC (Figure 5). After fixing the tissue an antigen retrieval step was performed. Alpha synuclein labelling was performed with either a rabbit anti-pSer129 monoclonal antibody or with a pool of serum from monkeys immunized with ACI-7104.056. Images were acquired with a Panoramic Scan P150 (3D HISTECH) and analysed with the software CaseViewer (3D HISTECH).

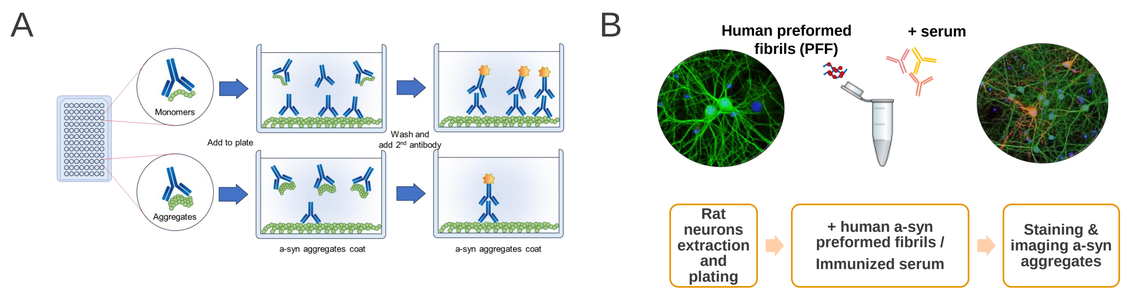


***Figure 2:** Schematic representation of the assay set-up. (A) Competitive inhibition assay; (B) Seeding assay using primary rat neurons*

ACI-7104.056 induces a strong IgG response recognizing pathological a-syn aggregates

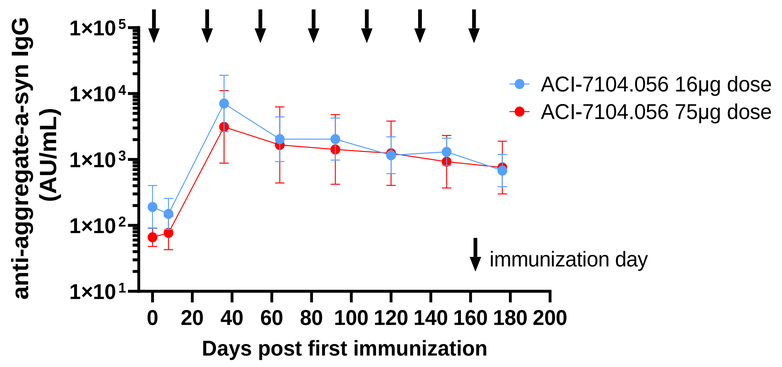


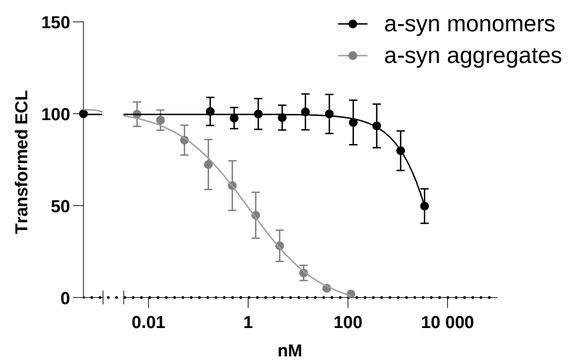
***Figure 3:** Anti-aggregate-a-syn IgG titers in the serum of monkeys (n=10 per group) at pre-dose and following immunization with ACI-7104.056 at two different doses. Data are expressed as geometric mean with 95% confidence interval (CI) per group.*

Results

ACI-7104.056 induces a strong IgG response recognizing pathological a-syn aggregates

In competitive inhibition experiments, IgGs generated by immunization of NHPs with ACI-7104.056 gave a >3-log differential for aggregated over monomeric species of a-syn

A



B

	IC50 Average	Ratio IC50 monomers/ IC50 aggregates
IC50 nM Monomers	>3457	>2784
IC50 nM aggregates	1.24	

***Figure 4:** Monkey immune sera (day 120 post 1st immunization) were incubated with increasing concentration of a-syn aggregates or monomers and subsequently analyzed in a MSD plate precoated with a-syn aggregates. A) The average percentage of signal (n=7 monkeys) is normalized against the untreated samples (without competition), Data are expressed as the mean ± SD; B) IC50 values of inhibition was calculated and used to obtain the monomers vs aggregates ratio.*

ACI-7104.056-induced antibodies bind to a-syn pathology in PDD(1) and MSA(2) brain slides

(1) Parkinson's Disease Dementia; (2) Multiple System Atrophy

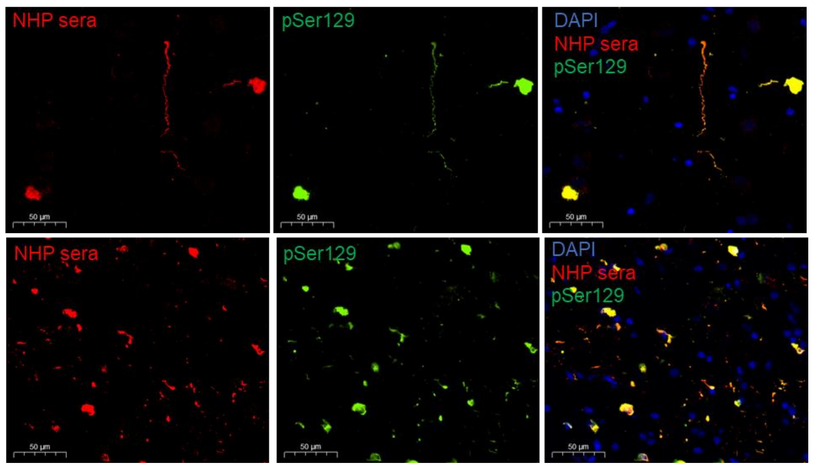


***Figure 5:** A pool of monkey immune sera (day 36 post 1st immunization) was used to label a-syn in frozen brain sections of A) PDD or B) MSA in co-labeling with an pSer129 antibody. (DAPI in blue; sera from immunized monkeys in red; pSer129 in green).*

ACI-7104.056-induced antibodies inhibit intracellular aggregation in a neuronal seeding assay

Serum of immunized monkeys show an inhibitory effect on the accumulation of a-syn aggregates in a seeding assay using primary rat neurons.

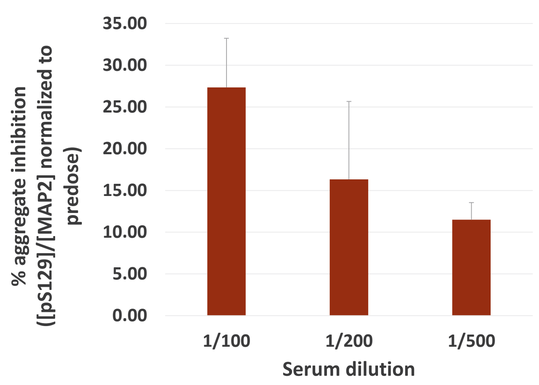


***Figure 6:** Serum collected on day 36 post 1st immunization leads to dilution-dependent reduction of a-syn aggregates within neurons as compared to diluted serum collected from the same animals before immunization (pre-dose).* Data are expressed as the mean ± SEM.

Conclusion

- ACI-7104.056 is safe and well tolerated in monkeys. Transient injection site reactions observed as expected
- A strong IgG response against a-syn aggregates observed after two immunizations and maintained at a high level until study end
- Elicited antibodies bind a-syn aggregates with high specificity and bind to pathological a-syn present in brain tissue from patients
- Antibodies reduce the number of intracellular a-syn aggregates in a primary neuronal seeding assay demonstrating efficient blockage of a-syn propagation