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Improved brain expression of anti-amyloid β scFv by complexation of mRNA including a secretion sequence with PEG-based block copolymer

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Abstract: Amyloid β aggregates have been proposed as central effectors in Alzheimer's disease, with several passive immunization clinical trials. We used mRNA encoding oligomer-specific anti-amyloid β (A β) scFv for passive immunotherapy. These scFvs could recognize amyloid oligomers with significant affinity, delay the aggregation of amyloid β *in vitro*, and dissociate A β aggregates *in vitro*. Intra-cerebroventricular injection of pDNA and mRNA encoding the scFv resulted in detectable scFv levels in the brain, with higher *in vivo* expression from mRNA. Notably, injection of mRNA decreased A β burden in an acute amyloidosis model. scFv production *in situ* from mRNA represents a novel immunotherapy approach, and a safer alternative compared to the administration of anti-A β antibodies or adenovirus encoding antibodies.

Keywords: Alzheimer's disease, mRNA, passive immunotherapy, scFv, smart copolymer, amyloid beta.

1. INTRODUCTION

Alzheimer's disease (AD) is a common form of age-related dementia, and the fifth leading cause of death of the elderly.(1) AD is characterized by cognitive impairment due to loss of neuronal function, correlated with higher A β cerebral levels and aggregation of toxic A β oligomers into fibrils and senile plaques, accompanied with the aggregation of Tau protein in neurofibrillary tangles. (2, 3) 36 million people were diagnosed with AD in 2010 and its incidence is expected to increase by 300% by 2050, urging for the development of an effective therapy.(4) Several strategies have been proposed against AD among which inhibition of the production of amyloid beta and destruction of senile plaques of aggregated A β .(5) Immunotherapy either active (vaccination with A β oligomers to induce an active immune response) or passive (vaccination with anti-A β antibodies) aims at either activating A β clearance, reducing senile plaques, or slowing down the formation of A β aggregates to prevent further growth and toxicity. (5, 6) Both active and passive immunotherapy has shown sufficient successes to reach Phase II or III clinical trials.(2, 6) Nevertheless, in all these trials, the antibodies are intravenously injected requiring

chronic dosing and high quantities; thereby increasing the probability of adverse effects and costs of therapy. Moreover, although Bapineuzumab anti-A β monoclonal antibody (mAb) was detected in the hippocampus of transgenic mice 7 days after a single intravenous injection and a 15-25 % decrease in A β was detected in patients, no cognitive benefit was obtained suggesting a threshold effect.(7, 8) Further support for insufficient antibody delivery to the brain came for Bapineuzumab and Solanezumab Phase III clinical trials failures with decreased brain A β and hyper phosphorylated Tau levels without cognitive improvement.(9-11) In addition, Bapineuzumab caused dose-limiting vasogenic edema.(12) It should be noted that these dose limiting side effects could be attributed at least in part to the mechanism of anti-A β antibodies: binding to senile plaques is followed by endocytosis of immune complexes by microglia *via* the constant fragment receptor (Fc γ R), with brain edemas related to inflammation induced by engagement of Fc γ R.(13, 14)

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Whereas use of single-chain variable fragment (scFv) lacking constant regions have been proposed to address this treatment-related inflammation, current approaches are limited to delivery of anti-A β scFv using adeno-associated virus (AAV) for high *in situ* expression of the antibody, which led to improved cognitive functions in AD mice models.(15, 16)

These results appear consistent with the high levels of senile plaques in patients, (3, 17, 18) and suggest improved delivery of oligomer-specific antibodies without constant fragment, as a critical parameter for AD immunotherapy. However, use of AAV led to sustained expression of scFv in the brain, resulting in detrimental cerebrovascular damage and brain hemorrhages.(19)

We introduce anti-A β scFv secretion *in situ* after mRNA injection as an alternative to chronic mAb dosing or viral gene therapy. Our strategy combines mRNA for acute expression of the protein even in quiescent cells such as neurons without the integration risks associated with DNA, and a secretion sequence for *in vivo* production of the scFv irrespective of the cell type transfected.(15, 20, 21) In this study we describe the brain expression of anti-A β scFvs from DNA and mRNA after intracranial injections. We demonstrated that complexation of mRNA encoding A β ₁₋₄₂ fibril-specific scFv (22, 23) with the polyethyleneglycol-poly[N9-[N-(2-aminoethyl)-2-aminoethyl]aspartamide] (PEG-PAsp(DET)) smart block copolymer (24, 25) resulted in higher scFv levels over DNA constructs both in primary neurons and *in vivo*. Addition of a secretion signal further increased the scFv levels and suggested therapeutic activity in an acute amyloidosis model.

2. MATERIALS AND METHODS

2.1. Materials

A β ₁₋₄₀, A β ₁₋₄₂, anti-A β ELISA kits, mouse anti-6xHis-tag antibody, anti-mouse HRP-conjugated antibody were purchased from Wako. Passive Lysis Buffer was ordered from Promega. Anti-6xHis-tag ELISA kit was from Genscript. pFUSE2ss-CLIg-hl2 plasmid was from Invivogen. Human Embryonic Kidney cells expressing the T7 polymerase (HEK 293-T7) were a kind gift from Pr. Huang and Pr Midoux.(26)

2.2 Preparation of fibrillar A β

A β ₁₋₄₂ protofibrils were obtained by mixing A β ₁₋₄₂ in DMSO with DMEM media, as in (27). Aggregation status was verified by electron microscopy.

2.3. Production of recombinant scFv

The DNA sequences encoding the scFvs of B6 and B7 are described in (22). The DNA of 3D6 scFv was chemically synthesized by Genscript and the sequence was optimized for expression in *E. coli*. Detailed methods are described in the Supplementary Material. Briefly, the genes encoding scFv with N-terminal pel-B leader sequence and C-terminal hexahistidine tag were introduced into BL21(DE3) and the proteins were expressed either in lysate (B6, B7 and control scFv) or in culture medium (3D6)(28). Afterwards, the scFvs were purified by immobilized metal-affinity chromatography followed by size exclusion chromatography.

2.4. Plasmid construction and mRNA preparation

pCMV-IL2ss-scFv
EcoRI and NheI sites were added to the sequences of the scFv by PCR. Then EcoRI-scFv-NheI was inserted in fusion with the IL2 signal sequence (IL2-ss) in the multiple cloning

site of pFUSE2ss-CLIg-hl2 to obtain the pCMV-IL2ss-scFv construct.

pT7CMV-IL2ss-scFv-120A

Sequences form the T7 promoter and the 120 poly-adenosine tail were extracted from pSP73-polyA120 by PCR and cloned in the pCMV-IL2ss-scFv plasmid to obtain the pT7CMV-IL2ss-scFv-120A plasmids.(29) These plasmids were linearized with BsmBI and used for mRNA preparation with a mMessage mMachine T7 kit (Ambion), as previously reported.(29, 30) mRNA concentration was determined by absorbance at 260 nm and its integrity analyzed by capillary electrophoresis (Agilent Bioanalyzer).

2.5. Affinity analysis

Binding of the scFvs to A β was determined by surface plasmon resonance (Biacore T200). Briefly, various conformations of A β were immobilized on CM5 sensor chip by amide coupling, and scFv was injected at varied concentrations. The sensorgrams were globally fitted to obtain kinetic parameters. For detail, see the Supplementary Materials.

2.6. Electron microscopy

Aggregation status of the A β used to coat the ELISA plates and sensor chips was verified by electron microscopy. The morphology of A β was observed using the H-7000 transmission electron microscope (TEM) (Hitachi Ltd., Tokyo, Japan) operated at an acceleration voltage of 75 kV, as reported previously.(31) Glow-discharged copper TEM grids (Nisshin EM Corp., Japan) were coated with A β and 2% uranyl acetate for staining. TEM images were analyzed using Image J.35

2.7. Preparation of primary neurons

Primary neurons were isolated from day 16 pregnant C57BL/6J mice according to (32). Neurons were further purified using the Nerve-Cell Culture System kit (Sumitomo), seeded onto collagen-coated 24-well plates and transfected 3-weeks after isolation.

2.8. In vitro transfection and Western blot

HEK 293-T7 cells were cultured in DMEM containing 10% fetal bovine serum (FBS). Cells were transfected with the constructs using Lipofectamine LTX following manufacturer's protocol. Cells were also transfected with the PEG-PAsp(DET) copolymer. PEG-PAsp(DET) polyplexes were formed as in our previous study (29). After transfection, Western blot was performed according to.(33) Briefly, cell culture supernatant and lysate were run on a SDS-PAGE gel and transferred to a PVDF membrane before probing with an anti-6xHis-tag antibody, anti-A β antibody, or β -actin as loading control followed by HRP-conjugated secondary antibody and developing with an ECL chemiluminescence kit (GE Healthcare).

2.9. In vitro cytotoxicity

Cytotoxicity was evaluated performing an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as in.(33)

2.10. Anti-6xHis-tag ELISA

scFv levels were determined using a competitive 6xHis-tag ELISA kit following manufacturer's protocol (His tag ELISA detection kit, Genscript).

2.11. Amyloid β capture ELISA

A protocol similar to (33) was used. ELISA plates (Nunc Immuno Micro 96-well plates) were coated with soluble or aggregated A β 1-40 and 1-42 in TBS (Tris-

buffered saline, pH 7.4) at 40 µg/mL for 1h at RT. Wells were washed with TBS-T (TBS containing 0.05% Tween 20) and blocked with TBST containing 1% bovine serum albumin (TBS-T/BSA) for 1h at RT. Wells were incubated with 10 µg/mL scFv in TBS-T/BSA for 2h at RT, followed by incubation with HRP-conjugated anti-mouse antibody for 1h at RT. Signal was revealed using TMB (3,3',5,5'-tetramethylbenzidine) and absorbance was measured at 450 nm after addition of a stop solution (0.16 M sulfuric acid).

2.12. Thioflavine T (ThT) assay

A ThT assay was performed to estimate the impact of the scFv on Aβ aggregation.(34) Briefly, Aβ aggregates and scFv were diluted in PBS at 1:1 and 10:1 scFv to Aβ molar ratios. 1 µM ThT was included to monitor aggregation status over 16h at 30°C at an excitation of 440/5 nm and an emission of 480/5 nm.

2.13. Intracranial injections

C57BL6/J female mice (8-10 weeks) under 2.5% isoflurane anesthesia were intracerebroventricularly injected in the lateral ventricle with DNA and mRNA polyplexes 2 mm caudal to bregma, 2 mm lateral to midline and 2.5 mm ventral.(29) Injection was performed with a Hamilton syringe with a 30G needle mounted on a SR-9M-HT stereotaxic apparatus (Narishige). Needle was slowly withdrawn before suturing.

2.14. Acute amyloidosis model

Aβ infusion was used as an AD model. (35) 2 µg of Aβ oligomers prepared as in (27) were injected intracerebroventricularly under anesthesia. 24h after, nucleic acid polyplexes encoding the corresponding scFv were injected at the same site. Brains were collected 24h after nucleic acid injection.

2.15. Transgenic AD model

C57BL6/J mice harboring Amyloid precursor protein (*App*) knock-in with Swedish/Iberian/Arctic mutations (*App*^{NL-G-F/NL-G-F} mice, (36)) were obtained from Riken BioResource Center(Tsukuba, Japan).

2.16. Quantitation of Aβ levels

Aβ was extracted from brains according to manufacturer's protocol.(37) Briefly, brains were homogenized in 2 mL Passive Lysis Buffer / g of brain and incubated for 20 min at 4 °C. Then lysates were centrifuged at 10,000 g for 10 min at 4 °C. Supernatants were discarded and pellets were resuspended in 1 mL / g of 70 % formic acid, transferred into ultracentrifugation tubes. Aggregates were cleared by ultracentrifugation for 6h at 120,000 g at 4 °C and used for anti-Aβ ELISA.

3. RESULTS AND DISCUSSION

3.1. Specificity of the scFv towards Aβ fibrils

We used 4 scFvs in our study (B6, B7, 3D6, and Control). B6 and B7 are recently described conformer-specific anti-Aβ₁₋₄₂ scFv identified by phage display (22, 23). 3D6 binds both to soluble and insoluble Aβ, with its humanized form bapineuzumab under clinical trials (7, 10, 38). The control scFv (further abbreviated as Con) has no specificity towards Aβ. We first performed *in vitro* experiments to confirm the ability of the scFv to bind to Aβ. To compare the affinity of these antibodies towards Aβ conformers, we performed surface plasmon resonance (SPR) experiments using chips coated with Aβ₁₋₄₂ of soluble monomer, soluble oligomer, and fibril (Table 1: see Figs S1-

S10 and Table S1 of the Supplementary Materials for the sensorgrams, fitting curves and kinetic parameters).

Table 1: Affinity of scFvs to various form of Aβ determined with surface plasmon resonance.

<i>K_D</i> (nM)	Monomers	Oligomers	Fibers
B6	104	24	52
B7	265	60	30
3D6	0.48	0.50	0.32

Among 4 scFvs, Con showed no significant binding. B6 and B7 demonstrated more than two fold specificity towards oligomers and fibers over monomers, in range with the 3-fold and 5-fold fiber specificity earlier reported for B6 and B7, respectively. (22) Notably, B6's affinity towards Aβ₁₋₄₂ fibers was as high as *K_D* = 52 nM, a 2-fold selectivity over monomers (104 nM). Preferential binding was even more selective for B7 scFv, with an 8-fold *K_D* decrease from monomer to fibers (265 nM to 30 nM), reaching a *K_D* lower than the 50 nM reported for previous report of Aβ-specific scFvs.(39, 40) On the other hand, 3D6 bound to conformations of Aβ without obvious specificity despite its strong binding compared to B6 and B7.

3.2. Disaggregation of preexisting Aβ fibrils

Since prevention of Aβ deposition and reduction of existing senile plaques are the focus of AD passive immunotherapy (5), we determined the capacity. To test the ability of our scFv to reduce existing fibrils as a model of senile plaque clearance, we incubated preformed Aβ₁₋₄₂ fibers with our scFvs (Fig. 1). Whereas fibrillary morphology was maintained after incubation with PBS or a control scFv, 3D6 and B7 scFv were able to disaggregate pre-formed fibrils. In contrast, B6 failed to disaggregate fibrils probably because of its lower affinity towards Aβ fibrils over soluble oligomers (52 nM vs 24 nM).

3.3. scFv secretion after transfection in cellulo

Next, we expressed the scFvs from DNA and mRNA constructs containing the interleukin 2 secretion sequence (IL2ss, Invivogen), optimized for the production and secretion of antibodies in mammalian cells.(21, 41) Nucleic acids were complexed with the smart PEG-PAsp(DET) copolymer to protect them from nucleases, and increase their stability in physiological fluids, resulting in detectable brain expression (25, 29). This copolymer was finely tuned to achieve pH-sensitive protonation in endosomal compartments after endocytosis, a protonation resulting in efficient endosomal escape of complexed nucleic acids without detectable cytotoxicity and high transgene expression both in cell lines and brain tissue.(29, 42, 43)

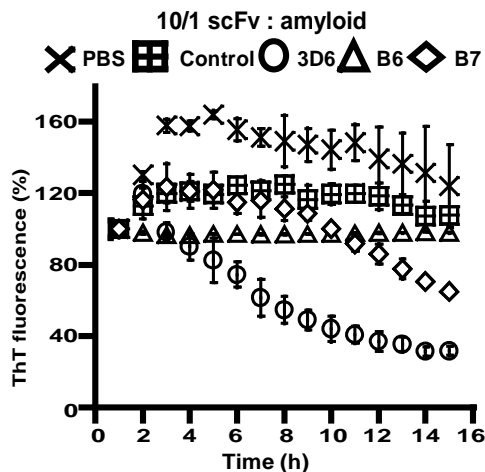


Figure 1: Disaggregation of preexisting $A\beta_{1-42}$ fibers. Disaggregation of preformed $A\beta_{1-42}$ fibrils in the presence of the scFvs. Data are expressed as the mean \pm S.D. (n = 3). * p < 0.05 by Student's t test compared to PBS. n.s. no statistical difference.

Moreover, PEG-PAsp(DET) is particularly suited for nucleic acid delivery in the brain as it was designed to rapidly degrade into nontoxic units to minimize tissue injury. (44, 45) Complexation of mRNA with the polymer formed spherical polyplex micellar nanoparticles (Fig. 2F) of 41 ± 10 nm with a neutral zeta potential of 0.9/4.8 mV due to the formation of hydrophilic shell of PEG surrounding the core of mRNA condensed with PAsp(DET) segments. scFv were detected as a 30 kDa band both in the cell culture media and in the cell lysate of cancer cells after DNA and mRNA transfection without significant decrease in cell viability (Fig. 2 A-D).

After demonstrating expression in cancer cell lines, we moved to primary neurons as a more relevant physiological model. Indeed, transfection of neurons and achieving therapeutic concentrations of agents in the brain are central problem in gene therapy applied to the brain. (29, 46) Use of mRNA or AAV vectors with secretion signals has been proposed to address this challenge. (29, 41, 46, 47) The advantage of the IL2ss signal addition was observed in primary neurons with 3.4-fold higher secretion of B7 scFV 24h after transfection with polyplexes formed with B7 mRNA with IL2ss over B7 mRNA devoid of secretion sequence, supporting the advantage of the secretion signal for antibody production in neurons (Fig. 2E). However, transfection efficiency was lower than that achieved with Lipofectamine LTX lipoplexes. Nevertheless, use of Lipofectamine LTX is limited by its high toxicity. (29)

3.4. Enhanced expression of mRNA with secretion sequence in the brain

mRNA encoding the scFv were injected intracerebroventricularly after complexation with PEG-PAsp(DET) to form polyplex micelles. At 4h and 24h after injection, we observed a notable benefit of mRNA over DNA for the transfection of brain cells with 3 to 11-fold

3.5. Therapeutic potential of the platform in an acute amyloidosis model

After confirming scFv expression in the brain, especially in neurons, we evaluated the therapeutic potential of our platform in an acute amyloidosis model (Fig. 4).

greater scFv expression for the three antibodies at 24h (Fig. 3). In contrast, expression from DNA was stable during the 4h-48h period, demonstrating acute expression after mRNA administration. Furthermore, in accordance with our *in cellulo* data in primary neurons, higher *in vivo* scFv levels were achieved with B7 mRNA containing the IL2ss over non-secreted B7 mRNA (Fig.3B, Fig. 3C).

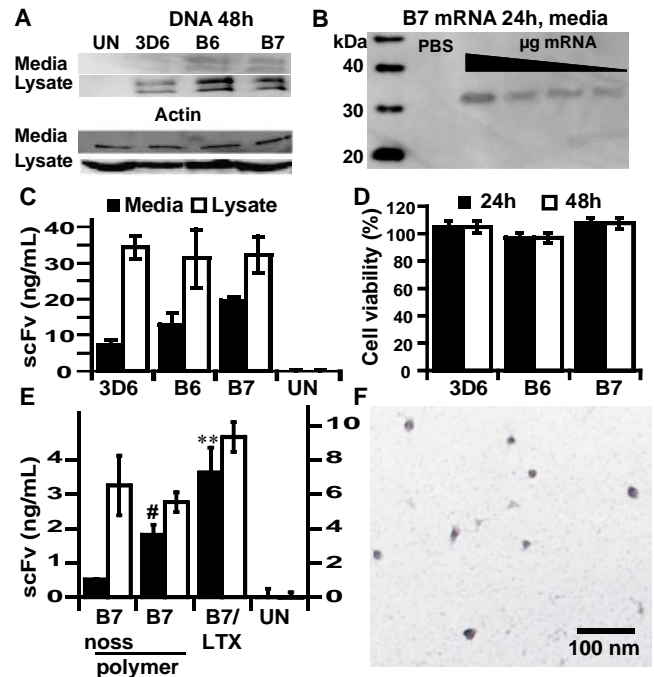


Figure 2: scFv expression *in cellulo*. A) scFv expression in cancer cells 48h after DNA transfection detected by immunoblot; B) scFv expression in cancer cells 24h after transfection with increasing amounts of B7 mRNA (2; 1; 0.5; 0.25 μ g) polyplexes; C) scFv expression in cancer cells at 24h detected by ELISA; D) Cell viability after cancer cell transfection; E) scFv expression in primary neurons 24h after mRNA transfection; F) Morphology of mRNA polyplexes by TEM. Data are expressed as the mean \pm S.D. (n = 3). Student's t test was performed, ** p < 0.01 compared to polyplexes-treated neurons, # p < 0.05 compared to neurons treated with B7noss mRNA polyplexes. UN, untransfected cells.

These transgenic mice overproduce both App and several $A\beta$ peptide fragments, an overproduction which leads to AD-like symptoms. Use of this model was crucial to confirm engagement of pathological $A\beta$ by the scFv, rather than of co-injected *in vitro* prepared $A\beta$ fibrils (Fig. 5).

In this more stringent AD model, no decrease in formic acid-soluble $A\beta$ was measured after injection of B7-IL2ss mRNA polyplexes.

Whereas the injection of DNA encoding scFv did not result in a decrease of $A\beta$ levels except for 3D6, injection of mRNA resulted in 40 % decrease in an acute amyloidosis model (Fig. 4). Superior decrease of $A\beta$ levels after mRNA injection over DNA was correlated with the higher scFv

expression after mRNA administration (Fig. 3). In contrast, no A β decrease was detected with control scFv mRNA.

3.6. Therapeutic relevance in a transgenic AD model

Finally, we used a transgenic AD model to confirm the relevancy of our IL2ss mRNA polyplex platform for Alzheimer’s disease treatment (Fig. 5). We used the single App knock-in model App^{NL-G-F/NL-G-F} harboring three familial mutations in the App gene.(36)

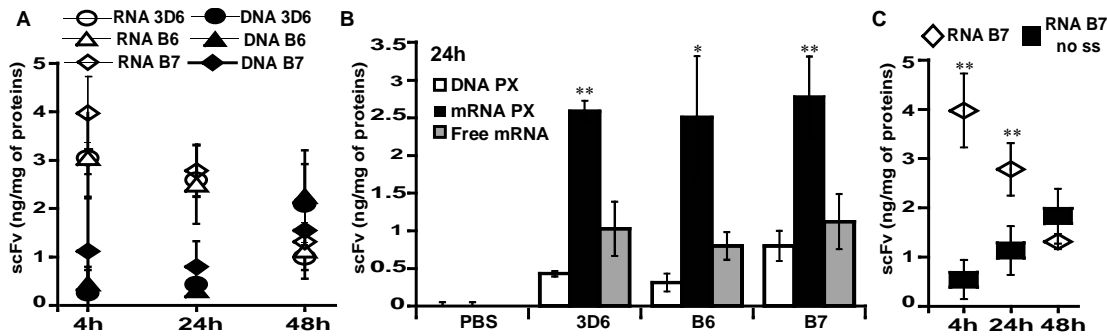


Figure 3: scFv expression in vivo after intracranial injection. A) Kinetics of scFv expression in the brain 4h, 24h and 48h after injection of DNA and mRNA polyplexes detected by ELISA; B) *In situ* expression of DNA and mRNA polyplexes or free mRNA at 24h post-injection; C) *In situ* expression of B7 mRNA polyplexes with and without IL2ss by ELISA. Data are expressed as the mean \pm S.D. (n = 5). * p < 0.05, ** p < 0.01 by Student’s t test compared to PBS.

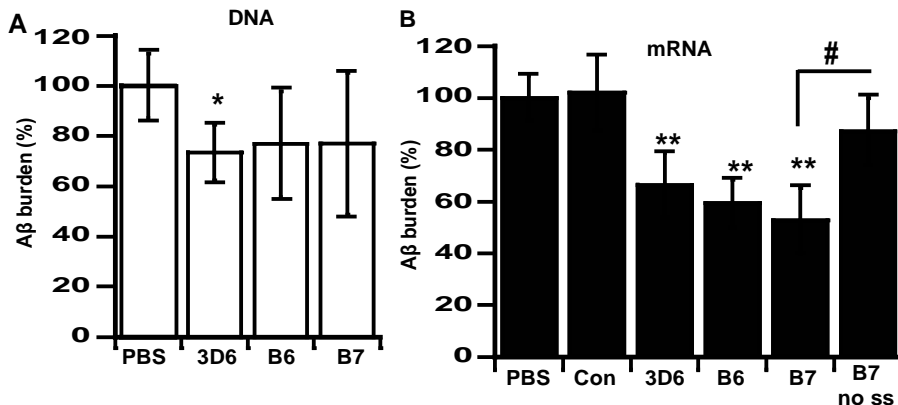


Figure 4: Decrease of fibrillary A β levels after DNA and mRNA injection in an acute amyloidosis model. DNA (A) and mRNA (B) polyplexes were intracranially injected in an acute amyloidosis mouse model. Aggregated A β levels were quantitated 24h post-injection by anti-A β ELISA. Data are expressed as the mean \pm S.D (n = 5). * p < 0.05, ** p < 0.01 by Student’s t test compared to PBS.; # p < 0.01 compared to B7 no ss mRNA treatment.

intracranially injected in App^{NL-G-F/NL-G-F} mice. Aggregated A β levels were quantitated 24h post-injection by anti-A β ELISA. Data are expressed as the mean \pm S.D (n = 4).

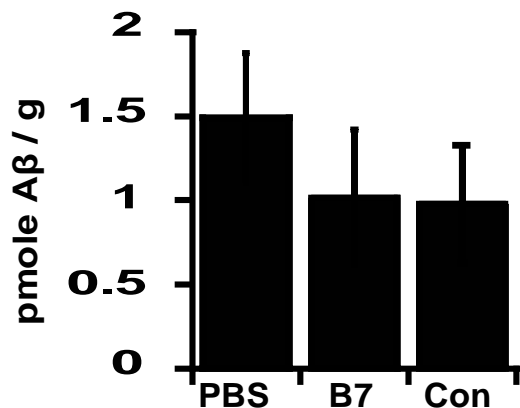


Figure 5: Therapeutic potential of mRNA polyplexes in a transgenic AD model. PBS or mRNA polyplexes were

CONCLUSION

The scFvs were able to bind to A β aggregates with high affinity. In accordance with previous studies, B7 scFv had affinity towards A β fibers and was able to disaggregate preformed aggregates *in vitro* (40, 48-50). Intracerebroventricular injection of mRNA and DNA polyplexes encoding the scFvs achieved detectable antibodies levels in the brain. Twenty four hours after injection, highest levels of scFvs were measured when they were delivered as mRNA polyplexes rather than DNA polyplexes or free mRNA. This demonstrates the benefit of using mRNA for brain expression of therapeutic nucleic acids and the requirement for improved mRNA nanocarriers to achieve mRNA expression in brain tissue.(29) Superior expression in the brain could be attributed to the PEG-PAsp(DET) copolymer which, in addition to protecting mRNA from early degradation, alters its intracellular trafficking to result in detectable expression in neurons *in vivo*.(25, 29, 43) To further augment the intracranial expression of administered mRNA, the IL2ss was fused to the scFv.(21) Addition of IL2ss resulted in higher brain scFv concentration after injection of B7-IL2ss mRNA over B7 mRNA.

Notably, B7-IL2ss mRNA encoding scFv delivery was able to decrease A β burden in an acute amyloidosis model. The finding that only the construct with a secretion sequence was able to decrease A β burden suggests that a threshold concentration of extracellular anti-A β is required to engage A β deposits and promote their disaggregation, a pattern similar to other anti-A β_{1-42} scFvs.(51, 52) In agreement with our results, Levites and coworkers reported preferential A β fibrils binding of 3H3 scFv fused to a secretion signal (pSecTag-3H3 scFv) over un-secreted 3H3, with anti-A β fibrils OD values of 0.354 and 1.089, respectively.(52)

In this study, we used two AD models reflecting early pre-symptomatic AD (acute amyloidosis) and a transgenic model with behavioral impairments similar to those observed at clinical presentation.(5, 27, 36) Although a detectable decrease in A β burden could be measured in the early AD model, no reduction in A β_{1-42} levels could be achieved after intracranial injection of the polyplexes in the AD transgenic mouse model. Such contrasting results should be interpreted in light of the stabilization of amyloid aggregates and fibers by other proteins such as Tau with age, a stabilization negligible in the acute AD model.(5, 6) Data from Mamikonyan *et al.* further support activity of our platform only in early stages of AD. They developed an anti-A β_{1-42} antibody which despite being able of A β fibrils disaggregation *in vitro* and, expression in the brain after intra-hippocampal injection, failed to induce a decrease in amyloid burden in the 3xTg transgenic AD mouse model.(40)

Our report is in agreement with the reported increased production of antibodies with IL2ss and with the enhanced therapeutic activities of AAV expressing galanin or

neprilysin with secretion sequences.(21, 41, 47) Whereas intravenous administration of anti-A β relies on chronic administration to achieve therapeutic brain levels (5, 7, 9, 36), direct infusion of whole antibodies or antibody fragments has also proven effective in reducing A β levels in several AD models.(42, 44-46) However, these treatments face the limited (0.1 %) distribution of intravenous immunoglobulins to the brain with accumulation chiefly at the periphery of brain vessels resulting in edemas, and rapid *in vivo* degradation of infused antibodies.(5, 6)

Our approach challenges these limitations and we demonstrate that introduction of mRNA encoding A β oligomer-specific scFv can result in detectable antibody levels in the brain with successful transfection of neurons *in vivo* resulting in decreased A β burden in an acute AD mouse model but not a model reflecting later AD stages. These preliminary data suggest administration of anti-A β scFv encoding mRNA as a potential passive immunotherapy strategy to treat primary amyloidosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Minino AM, Xu J, Kochanek KD. Deaths: preliminary data for 2008. National vital statistics reports : from the Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System. 2010;59(2):1-52.
2. Samadi H, Sultzer D. Solanezumab for Alzheimer's disease. Expert opinion on biological therapy. 2011;11(6):787-98.
3. Svedberg MM, Hall H, Hellstrom-Lindahl E, Estrada S, Guan Z, Nordberg A, et al. [(11)C]PIB-amyloid binding and levels of Abeta40 and Abeta42 in postmortem brain tissue from Alzheimer patients. Neurochemistry international. 2009;54(5-6):347-57.
4. AsD I. World Alzheimer Report 2010. London: 2010.
5. Citron M. Alzheimer's disease: strategies for disease modification. Nature reviews Drug discovery. 2010;9(5):387-98.
6. Wisniewski T, Goni F. Immunotherapy for Alzheimer's disease. Biochemical pharmacology. 2014;88(4):499-507.

7. Bard F, Fox M, Friedrich S, Seubert P, Schenk D, Kinney GG, et al. Sustained levels of antibodies against Abeta in amyloid-rich regions of the CNS following intravenous dosing in human APP transgenic mice. *Experimental neurology*. 2012;238(1):38-43.
8. Novakovic D, Feligioni M, Scaccianoce S, Caruso A, Piccinin S, Schepisi C, et al. Profile of gantenerumab and its potential in the treatment of Alzheimer's disease. *Drug design, development and therapy*. 2013;7:1359-64.
9. Doody RS, Thomas RG, Farlow M, Iwatsubo T, Vellas B, Joffe S, et al. Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *The New England journal of medicine*. 2014;370(4):311-21.
10. Salloway S, Sperling R, Fox NC, Blennow K, Klunk W, Raskind M, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *The New England journal of medicine*. 2014;370(4):322-33.
11. Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, et al. Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet*. 2008;372(9634):216-23.
12. Salloway S, Sperling R, Gilman S, Fox NC, Blennow K, Raskind M, et al. A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. *Neurology*. 2009;73(24):2061-70.
13. Morgan D. The role of microglia in antibody-mediated clearance of amyloid-beta from the brain. *CNS & neurological disorders drug targets*. 2009;8(1):7-15.
14. Fuller JP, Stavenhagen JB, Teeling JL. New roles for Fc receptors in neurodegeneration-the impact on Immunotherapy for Alzheimer's Disease. *Frontiers in neuroscience*. 2014;8:235.
15. Levites Y, Jansen K, Smithson LA, Dakin R, Holloway VM, Das P, et al. Intracranial adeno-associated virus-mediated delivery of anti-pan amyloid beta, amyloid beta40, and amyloid beta42 single-chain variable fragments attenuates plaque pathology in amyloid precursor protein mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006;26(46):11923-8.
16. Ryan DA, Mastrangelo MA, Narrow WC, Sullivan MA, Federoff HJ, Bowers WJ. Abeta-directed single-chain antibody delivery via a serotype-1 AAV vector improves learning behavior and pathology in Alzheimer's disease mice. *Molecular therapy : the journal of the American Society of Gene Therapy*. 2010;18(8):1471-81.
17. Xia W, Yang T, Shankar G, Smith IM, Shen Y, Walsh DM, et al. A specific enzyme-linked immunosorbent assay for measuring beta-amyloid protein oligomers in human plasma and brain tissue of patients with Alzheimer disease. *Archives of neurology*. 2009;66(2):190-9.
18. Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P, et al. Correlation between elevated levels of amyloid beta-peptide in the brain and cognitive decline. *Jama*. 2000;283(12):1571-7.
19. Kou J, Kim H, Pattanayak A, Song M, Lim JE, Taguchi H, et al. Anti-Amyloid-beta Single-Chain Antibody Brain Delivery Via AAV Reduces Amyloid Load But May Increase Cerebral Hemorrhages in an Alzheimer's Disease Mouse Model. *Journal of Alzheimer's disease : JAD*. 2011;27(1):23-38.
20. Deering RP, Kommareddy S, Ulmer JB, Brito LA, Geall AJ. Nucleic acid vaccines: prospects for non-viral delivery of mRNA vaccines. *Expert opinion on drug delivery*. 2014;11(6):885-99.
21. Moutel S, El Marjou A, Vielemeyer O, Nizak C, Benaroch P, Dubel S, et al. A multi-Fc-species system for recombinant antibody production. *BMC biotechnology*. 2009;9:14.
22. Yoshihara T, Takiguchi S, Kyuno A, Tanaka K, Kuba S, Hashiguchi S, et al. Immunoreactivity of phage library-derived human single-chain antibodies to amyloid beta conformers in vitro. *Journal of biochemistry*. 2008;143(4):475-86.
23. Tanaka K, Nishimura M, Yamaguchi Y, Hashiguchi S, Takiguchi S, Yamaguchi M, et al. A mimotope peptide of Abeta42 fibril-specific antibodies with Abeta42 fibrillation inhibitory activity induces anti-Abeta42 conformer antibody response by a displayed form on an M13 phage in mice. *Journal of neuroimmunology*. 2011;236(1-2):27-38.
24. Osada K, Christie RJ, Kataoka K. Polymeric micelles from poly(ethylene glycol)-poly(amino acid) block copolymer for drug and gene delivery. *Journal of the Royal Society, Interface / the Royal Society*. 2009;6 Suppl 3:S325-39.
25. Miyata K, Nishiyama N, Kataoka K. Rational design of smart supramolecular assemblies for gene delivery: chemical challenges in the creation of artificial viruses. *Chemical Society reviews*. 2012;41(7):2562-74.
26. Li S, Brisson M, He Y, Huang L. Delivery of a PCR amplified DNA fragment into cells: a model for using synthetic genes for gene therapy. *Gene therapy*. 1997;4(5):449-54.
27. Stine WB, Jungbauer L, Yu C, LaDu MJ. Preparing synthetic Abeta in different aggregation states. *Methods in molecular biology*. 2011;670:13-32.
28. Choi JH, Lee SY. Secretory and extracellular production of recombinant proteins using *Escherichia coli*. *Applied microbiology and biotechnology*. 2004;64(5):625-35.
29. Uchida S, Itaka K, Uchida H, Hayakawa K, Ogata T, Ishii T, et al. In vivo messenger RNA introduction into the central nervous system using polyplex nanomicelle. *PloS one*. 2013;8(2):e56220.
30. Perche F, Benvegna T, Berchel M, Lebegue L, Pichon C, Jaffres PA, et al. Enhancement of dendritic cells transfection in vivo and of vaccination against B16F10 melanoma with mannosylated histidylated lipopolyplexes loaded with tumor antigen messenger RNA. *Nanomedicine : nanotechnology, biology, and medicine*. 2011;7(4):445-53.
31. Dirisala A, Osada K, Chen Q, Tockary TA, Machitani K, Osawa S, et al. Optimized rod length of polyplex micelles for maximizing transfection efficiency and their performance in systemic gene therapy against stroma-rich pancreatic tumors. *Biomaterials*. 2014;35(20):5359-68. Epub 2014/04/12.
32. Hilgenberg LG, Smith MA. Preparation of dissociated mouse cortical neuron cultures. *Journal of visualized experiments : JoVE*. 2007(10):562.

33. Perche F, Patel NR, Torchilin VP. Accumulation and toxicity of antibody-targeted doxorubicin-loaded PEG-PE micelles in ovarian cancer cell spheroid model. *Journal of controlled release : official journal of the Controlled Release Society*. 2012;164(1):95-102.
34. Liu R, Yuan B, Emadi S, Zameer A, Schulz P, McAllister C, et al. Single chain variable fragments against beta-amyloid (Abeta) can inhibit Abeta aggregation and prevent abeta-induced neurotoxicity. *Biochemistry*. 2004;43(22):6959-67.
35. Malm T, Ort M, Tahtivaara L, Jukarainen N, Goldsteins G, Puolivali J, et al. beta-Amyloid infusion results in delayed and age-dependent learning deficits without role of inflammation or beta-amyloid deposits. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;103(23):8852-7.
36. Saito T, Matsuba Y, Mihira N, Takano J, Nilsson P, Itohara S, et al. Single App knock-in mouse models of Alzheimer's disease. *Nature neuroscience*. 2014;17(5):661-3.
37. Hosoda R, Saido TC, Otvos L, Jr., Arai T, Mann DM, Lee VM, et al. Quantification of modified amyloid beta peptides in Alzheimer disease and Down syndrome brains. *Journal of neuropathology and experimental neurology*. 1998;57(11):1089-95.
38. Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, et al. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nature medicine*. 2000;6(8):916-9.
39. Nisbet R, Nigro J, Breheny K, Caine J, Hattarki M, Nuttall S. Central amyloid- β -specific single chain variable fragment ameliorates A β aggregation and neurotoxicity. *Protein Engineering Design and Selection*. 2013;26(10):571-80.
40. Mamikonyan G, Necula M, Mkrtichyan M, Ghochikyan A, Petrushina I, Movsesyan N, et al. Anti-A beta 1-11 antibody binds to different beta-amyloid species, inhibits fibril formation, and disaggregates preformed fibrils but not the most toxic oligomers. *The Journal of biological chemistry*. 2007;282(31):22376-86.
41. Haberman RP, Samulski RJ, McCown TJ. Attenuation of seizures and neuronal death by adeno-associated virus vector galanin expression and secretion. *Nature medicine*. 2003;9(8):1076-80.
42. Kanayama N, Fukushima S, Nishiyama N, Itaka K, Jang WD, Miyata K, et al. A PEG - Based Biocompatible Block Cationer with High Buffering Capacity for the Construction of Polyplex Micelles Showing Efficient Gene Transfer toward Primary Cells. *ChemMedChem*. 2006;1(4):439-44.
43. Miyata K, Oba M, Nakanishi M, Fukushima S, Yamasaki Y, Koyama H, et al. Polyplexes from poly (aspartamide) bearing 1, 2-diaminoethane side chains induce pH-selective, endosomal membrane destabilization with amplified transfection and negligible cytotoxicity. *Journal of the American Chemical Society*. 2008;130(48):16287-94.
44. Masago K, Itaka K, Nishiyama N, Chung U-i, Kataoka K. Gene delivery with biocompatible cationic polymer: pharmacogenomic analysis on cell bioactivity. *Biomaterials*. 2007;28(34):5169-75.
45. Itaka K, Ishii T, Hasegawa Y, Kataoka K. Biodegradable polyamino acid-based polycations as safe and effective gene carrier minimizing cumulative toxicity. *Biomaterials*. 2010;31(13):3707-14.
46. Karra D, Dahm R. Transfection techniques for neuronal cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2010;30(18):6171-7.
47. Carty N, Nash KR, Brownlow M, Cruite D, Wilcock D, Selenica ML, et al. Intracranial injection of AAV expressing NEP but not IDE reduces amyloid pathology in APP+PS1 transgenic mice. *PLoS one*. 2013;8(3):e59626.
48. Fukuchi K, Tahara K, Kim HD, Maxwell JA, Lewis TL, Accavitti-Loper MA, et al. Anti-Abeta single-chain antibody delivery via adeno-associated virus for treatment of Alzheimer's disease. *Neurobiology of disease*. 2006;23(3):502-11.
49. Wang YJ, Pollard A, Zhong JH, Dong XY, Wu XB, Zhou HD, et al. Intramuscular delivery of a single chain antibody gene reduces brain Abeta burden in a mouse model of Alzheimer's disease. *Neurobiology of aging*. 2009;30(3):364-76.
50. Murakami K. Conformation-specific antibodies to target amyloid beta oligomers and their application to immunotherapy for Alzheimer's disease. *Bioscience, biotechnology, and biochemistry*. 2014;78(8):1293-305.
51. Zhang Y, Yang H-Q, Fang F, Song L-L, Jiao Y-Y, Wang H, et al. Single Chain Variable Fragment Against A β Expressed in Baculovirus Inhibits Abeta Fibril Elongation and Promotes its Disaggregation. 2015.
52. Levites Y, O'Nuallain B, Puligedda RD, Ondrejcek T, Adekar SP, Chen C, et al. A Human Monoclonal IgG That Binds A β Assemblies and Diverse Amyloids Exhibits Anti-Amyloid Activities In Vitro and In Vivo. *The Journal of Neuroscience*. 2015;35(16):6265-76.