Poster #2046

ABL301, BBB-Crossing Trojan Horse Bispecific Antibody Specifically Targeting Aggregated a-synuclein for the Treatment of Parkinson's Disease (PD).

Sang Hoon Lee[1], Sungwon An[1*], Jinhyung Ahn[1], Jung-Won Shin[1], Dongin Kim[1], Jaehyun Eom[1], Yonggyu Son[1], Eunsil Sung[1], Sang Myun Park[2], You Ree Choi[2], Seo-Jun Kang[2]

[1] ABL Bio Inc., Seongnam, South Korea ; 2 College of Medicine, Ajou University, South Korea. *contact: sungwon.an@ablbio.com*

Abstract

Introduction. Alpha-synuclein (α-syn) is expressed mainly in presynaptic terminals in neurons, where it functions for synaptic release of neurotransmitters. In diseased brains with Parkinson's disease (PD) and other synuclein-related diseases (synucleinopathies), it is found as one of the major components of Lewy Bodies (LB), dense compact structures. As the disease progresses, more LBs appear in various brain areas, supporting the hypothesis of prion like, cell-to-cell transmission of α-syn aggregates. Facts including 1) α-syn as a major component of LB, 2) its gene dosage related to the severity of familial type of PD, and 3) toxicity of aggregated forms of α-syn in dishes and in animal models manifest the critical role of α-syn, especially its aggregated form in synucleinopathies.

Based on the findings above, various companies have been trying to develop antibody therapeutics against α-syn. However, their lead molecules have clear limitations. First, their antibodies barely cross blood-brain barrier (BBB). Indeed, it is well known that only 0.1~0.2 % of monoclonal antibodies injected is found in brains. Second, others' antibodies that are being developed either do not distinguish or only have limited distinction monomeric vs. aggregated α-syn. High preference of anti-α-syn antibodies to aggregated form is desirable because series of evidence indicates involvement of aggregated α-syn in diseases not the monomeric one.

ABL301 is a bispecific antibody (BsAb) composed of anti-α-syn IgG and anti-Target X-scFv acting as a BBB shuttle. ABL301 highly prefers aggregates to monomeric α-syn. Its preference was the best in various biochemical analyses among tested antibodies. It recognized human LB and Lewy neurites in postmortem brain tissues with PD. ABL301 has two mechanisms of action (MoA) regarding α-syn: 1) facilitation of microglial phagocytosis of extracellular α-syn and 2) Inhibition of its cell-to-cell transmission. ABL301's MoA is proved in multiple cell-based assays and *in vivo* test using transgenic mice expressing human α-syn. ABL301's dual MoAs differentiate it from competitors' antibodies in that they mainly focus on only inhibition of cell-to-cell transmission as their MoA.

ABL Bio's BBB shuttle is an antibody binding to a receptor (Target X) expressed on brain endothelial cells. Unlike other targets being used by others, the Target X is relatively highly expressed in brains compared to periphery. Moreover, its expression is relatively specific to brain endothelial cells not in normal brain cells, in contrast to high expression of transferrin receptor on normal brain cells. ABL Bio's BBB shuttle is bound to easily to therapeutic antibodies or peptides using amino-acid linkers. It enables therapeutics antibodies to go into brains using receptor-mediated transcytosis (RMT) by binding to the target X. It did not interfere with the Target X's ligand-mediated signaling/ cell proliferation nor changed the Target X's brain level after repeated systemic dosing.

ABL301 showed clearly increased BBB penetration than anti α-syn antibody. Its first generation (15XX clone) increased BBB penetration of therapeutic antibody in transwell assay system and *in vivo* test. ABL Bio pursued increase in BBB penetration by 1) affinity modulation of 15XX clone, and 2) Comparing valency of BsAb (bivalent or monovalent). The affinity variants showed augmented BBB penetration in human IPSC-derived transwell assay system, and increased CSF penetration in rats.

Conclusion. ABL301 is expected to have superior efficacy in PD and other synucleinopathy with its high specificity to α-syn aggregates and improved BBB penetration with its BBB shuttle. In the future, ABL Bio plans to conduct monkey studies with microdialysis to understand ABL301's BBB penetration In non-human primates and establish PBPK modeling based on the monkey data in order to anticipate Its BBB penetration in humans.

Structure & MoA

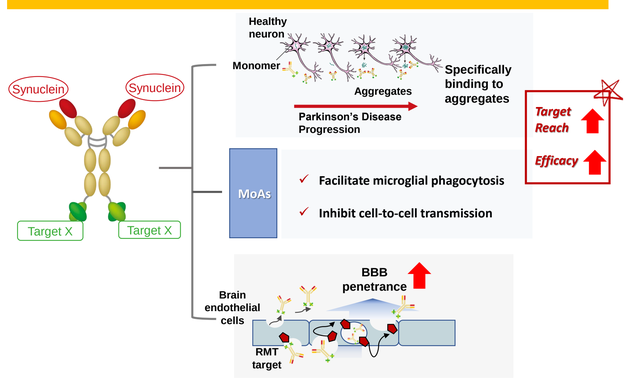
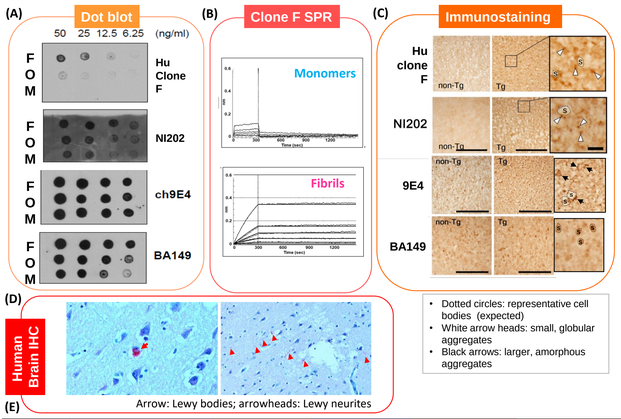


Fig. 1) ABL301's structure and its mechanisms of action. ABL301 is a bispecific antibody with anti-α-syn IgG and scFv targeting a receptor expressed on brain endothelial cells. Its highly specific targeting to aggregated α-syn and improved BBB penetration with a BBB shuttle will enable its high target reach, leading to better efficacy than competitors' antibodies. ABL301's anti-α-syn IgG has two major MoAs: 1) its facilitation of microglial phagocytosis of extracellular α-syn and 2) its inhibitory efficacy of cell-to-cell transmission of α-syn

Aggregate Preference



(E)

Clones	Dot blot			Octet_KD (10^{-10} nM)		SPR_KD (10^{-9} nM)		ELISA_EC$_{50}$ (nM)		IHC	
	Mon	Olig	Fibril	Mon	Fibril	Mon	Fibril	Mon	Fibril	LB, LN*	mThy-1 tg
Clone F	-**	Bind	Bind	-	1.60	-	0.287	-	2.177	✓	Small, globular aggre -gates
9E4 (Roche)	Bind	Bind	Bind	0.582	<0.01	N/A***	N/A	0.002	0.006	✓	Smeared, amorpho us
NI.202 (Biogen)	Weak bind	Bind	Bind	-	5.05	N/A	N/A	0.889	0.133	✓	Small, globular aggre -gates
BA149 (AbbVie)	Bind	Bind	Bind	0.731	0.61	N/A	N/A	0.096	0.053	✓	Smeared, located at soma

*: Lewy bodies, Lewy neurites; **: Not measurable; ***: Not available

Fig. 2) Clone F preferentially recognizes aggregated α-syn. (A) Dot blot images of ABL's lead α-syn IgG (hu clone F), Biogen/ Neurimmune's (NI202), Roche/ Prothena's (9E4) and AbbVie/ BioArctic's (BA149) α-syn IgGs with α-syn monomers (M), oligomers (O) and fibrils (F). (B) SPR profile of Clone F. (C) Fixed cortex section of mThy-1 hu α-syn transgenic mice immuno-stained with antibodies denoted (A). Clone F and NI202 decorate mostly globular, small aggregates located in neurites or neuropil, whereas 9E4 and BA149 recognize α-syn in soma. (D) Clone F recognizes Lewy bodies/neurites (red arrows/arrowheads) in postmortem brain tissues with PD. (E) Summary of binding properties of Clone F and competitors' α-syn antibodies. Competitor antibodies' binding to human LB/LN was written based on their literatures.

in vitro MoAs

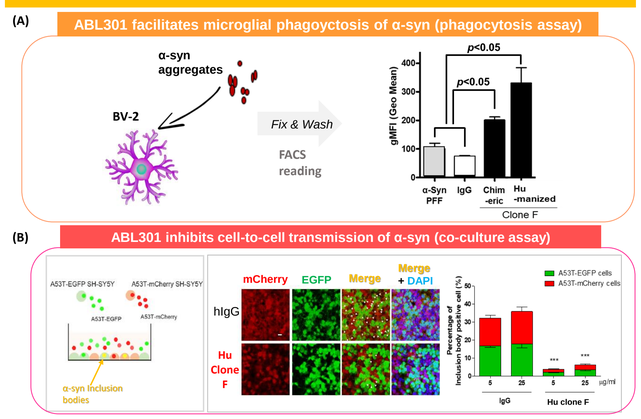
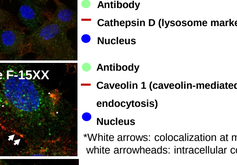


Fig. 3) Two MoAs of ABL301's anti-α-syn IgG.
(A) ABL's lead anti-α-syn IgG (clone F) facilitates uptake of extracellular α-syn aggregates by microglia. Chimeric and humanized clone F significantly increased the amount of α-syn uptaken by microglia (BV-2) compared to α-syn only and IgG-treated group. Statistical significance was estimated by student's t-test analysis.
(B) Humanized clone F significantly inhibited the production of inclusion bodies (yellow arrow) when two different cell lines were co-cultured (A53T α-syn-EGFP or A53T α-syn-mCherry-expressing SH-SY5Y cells) (Bars: % of inclusion body-positive mCherry cells (red) or EGFP cells (green), one-way ANOVA. *** *P < 0.001* against control IgG).

Superior Efficacy (*in vivo*)

(A)

	mThy-1 mouse
Promoter	Mouse neuronal protein (Thy-1)
Overexpress	Human α-synuclein
Inclusion bodies	O
Neuronal loss	O
Axon degeneration	O



Cortical pyramidal neurons and hippocampus of mThy-1 mouse

(B)

* HP: hippocampus

Fig. 4) Clone F improved neuropathology in PD animal model brain.
(A) Characteristics of mThy-1 human α-syn and its brain images stained with anti-α-syn antibody (image by *J. Neurosci. Res.* (2002) Rockenstein et al.).
(B) mThy-1 tg mice received IgG or clone F weekly with 10 mg/kg dose for 3 months. Non-tg littermates were used as controls. Representative brain images of non-transgenic littermates (WT) and transgenic mice (TG) with systemic treatments. Note that clone F-treated brains showed reduction of phosphorylated α-syn (marker for aggregated α-syn), increased NeuN immunoreactivity (marker for neurons, thus higher NeuN represents reduced neurodegeneration), reduced GFAP signal (marker for astrogliosis), and reduced Iba1 (marker for Proinflammatory cytokine) compared to IgG-treated brains (Clone A: another anti-α-syn antibody developed by ABL Bio; HP: hippocampus).

ABL BBB Target's Specificity

Proteomics data by paxdb.org

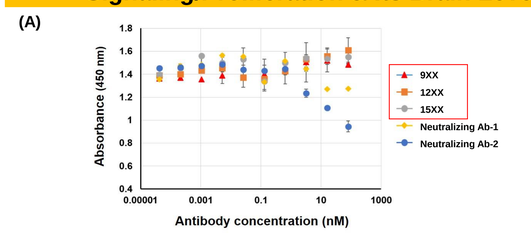
* Brain endothelial cells

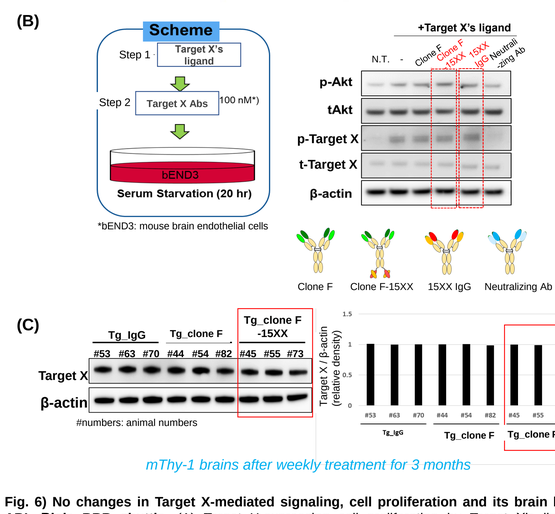
Fig. 5) ABL Bio's BBB target is highly specific to brain endothelial cells. (A) ABL's BBB target (Target X) is relatively highly expressed in brains than other periphery, compared to competitors' known targets (IR: insulin receptors; LRP1: LDL receptor-related protein 1; TfR: transferrin receptor).

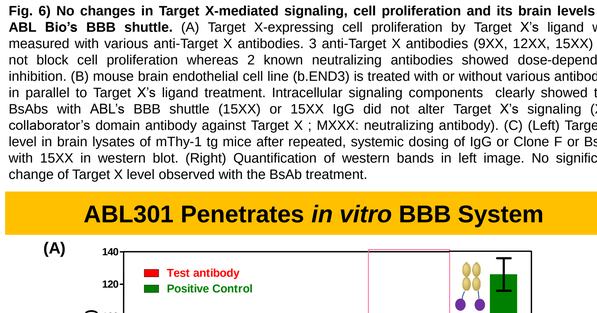
RMT-Mediated BBB Penetration

Fig. 6) RMT & ABL301's RMT mechanism (A) RMT mechanism of therapeutic antibody loaded with molecular Trojan horse (BBB shuttle). A molecular trojan horse is an antibody targeting a receptor expressed on brain endothelial cells. Its binding to the receptor induces internalization of the antibody-receptor Complex inside the brain endothelial cells. After being detached from the receptor, the therapeutic antibody with the BBB shuttle exits via early endosome or other exocytosis mechanism, reaching brain side. (B-D) ABL301 (Clone F-15XX)'s colocalization with endocytic markers but not with lysosomal markers in human microvascular brain endothelial cells indicates that ABL301 will enter the brains via RMT not being degraded inside cells.

15XX Induces No Changes in Target X-mediated Signaling/Proliferation & Its Brain Level



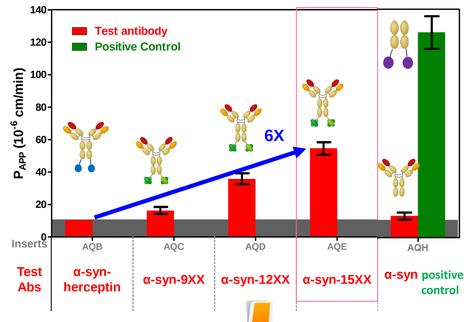




mThy-1 brains after weekly treatment for 3 months

Fig. 6) No changes in Target X-mediated signaling, cell proliferation and its brain levels by ABL Bio's BBB shuttle. (A) Target X-expressing cell proliferation by Target X's ligand was measured with various anti-Target X antibodies. 3 anti-Target X antibodies (9XX, 12XX, 15XX) did not block cell proliferation whereas 2 known neutralizing antibodies showed dose-dependent inhibition. (B) mouse brain endothelial cell line (b.END3) is treated with or without various antibodies in parallel to Target X's ligand treatment. Intracellular signaling components clearly showed that BsAbs with ABL's BBB shuttle (15XX) or 15XX IgG did not alter Target X's signaling (XX: collaborator's domain antibody against Target X ; MXXX: neutralizing antibody). (C) (Left) Target X level in brain lysates of mThy-1 tg mice after repeated, systemic dosing of IgG or Clone F or BsAb with 15XX in western blot. (Right) Quantification of western bands in left image. No significant change of Target X level observed with the BsAb treatment.

ABL301 Penetrates *in vitro* BBB System



Approaches to improve the BBB penetration of BsAb
✓ Affinity modulation of 15XX clone
✓ Test valency of BsAb (i.e. 2 scFvs or 1 scFv attached)

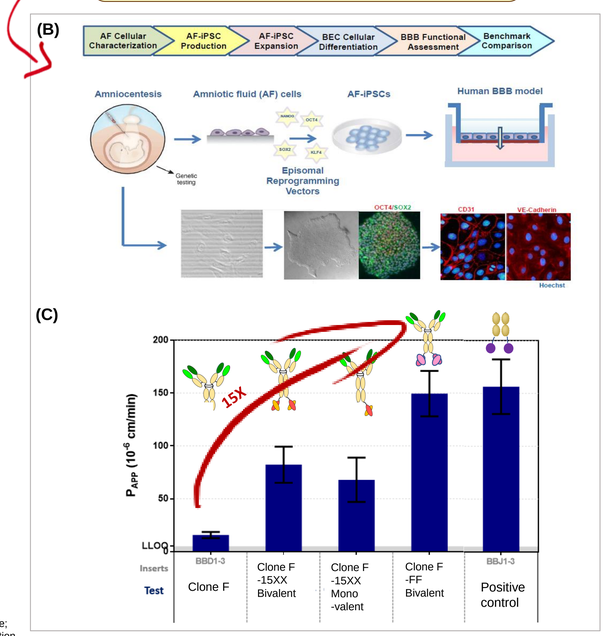
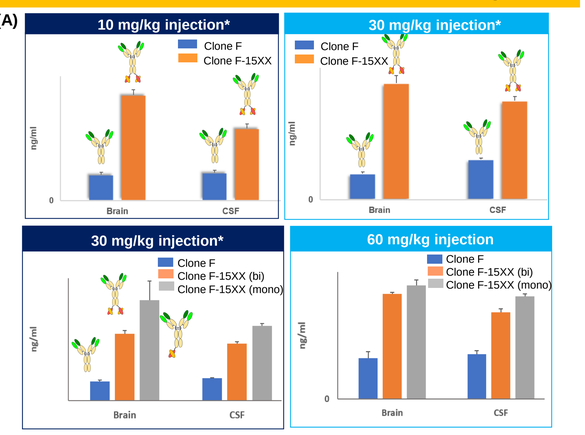
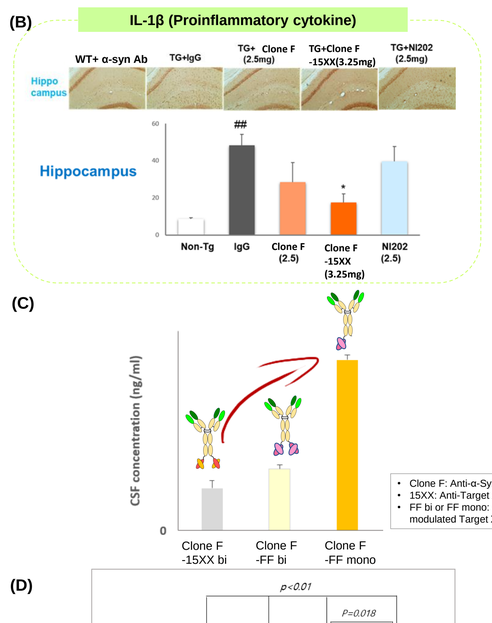


Fig. 7) BsAb penetrates *in vitro* BBB better than moAb. (A) *In vitro* BBB penetration of various antibodies in a transwell assay system using rat brain endothelial cells. BsAb with 15XX showed up to 6 times better penetration compared to anti-α-syn Ab or bispecific antibody with Herceptin scFv (negative controls) (α-syn: ABL's anti-α-syn antibody; 9XX, 12XX: ABL's anti-Target X antibodies; XX: collaborator's anti-Target X antibody). The second generation of BsAbs were generated in order to increase their BBB penetration: 1) affinity modulation of 15XX and 2) test their valency (i.e. 2 scFvs vs. 1 scFv). (B) Schematics of transwell assay using human iPSC-derived brain endothelial cells. The transwell assay using human brain endothelial cells showed higher tightness than rat cell based transwell assay. (C) BsAb with the 2nd generation BBB shuttles (i.e. affinity variants of 15XX) showed much higher BBB penetration than BsAb with 15XX *in vitro* in human iPSC-based transwell assay. Note that BsAb with FF variant showed about 15 times better penetration than clone F that is comparable to positive control (FF: affinity variant of 15XX clone).

Better BBB Penetration, Better Efficacy





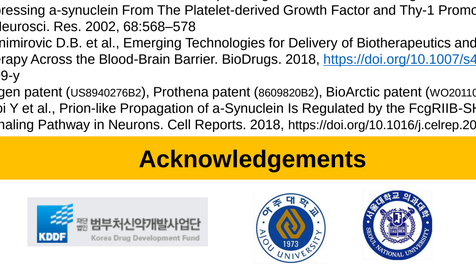


Fig. 8) BsAbs' better efficacy with their higher BBB penetration.
(A) (top) BsAb with 15XX showed up to 5 fold increase in brain penetration than clone F with 10 or 30 mg/kg injection to normal rats. Blood, brain and CSF of the the rats were analyzed 24 hrs after the injection using a sensitive mass analysis. (bottom) BsAb with 15XX showed up to 6 fold increase in brain penetration with 30 or 60 mg/kg in normal rats. Note that monovalent BsAb (i.e. one BBB shuttle attached to IgG) showed slightly better penetration than bivalent BsAb (i.e. two BBB shuttles). Based on the finding, The effect of BsAb valency on BBB penetration has been tested. (B) The efficacy of BsAb with 15XX was tested using the same experimental setup with 'Superior efficacy *in vivo*' section except lowering the dose as one forth (i.e. 2.5 mg/kg). BsAb clearly showed better efficacy in lowering IL-1β level compared to IgG, clone F and NI202 in hippocampus. BsAb showed overall superior efficacy in brain pathology compared to clone F and NI202 (data not shown). (C) BBB penetration of an affinity variant of 15XX clone (clones FF) were tested as BsAb (bivalent & monovalent) in normal rats. Monovalent BsAb with FF showed about 4-fold higher CSF penetration than bivalent BsAb with their parental 15XX after 48 hrs with 30 mg/kg injection in rats. (D) The efficacy of monovalent BsAb with FF compared to IgG and humanized clone F Was tested using mThy-1 tg mice with 4 times of antibodies within 8 days. Their brain lysates were analyzed using α-syn ELISA. Brain lysates from mice treated with monovalent BsAb with FF showed significantly lower α-syn level compared to IgG- or hu clone F-treated groups.

Conclusions

- ABL301 preferentially recognizes α-syn aggregates
- ABL301 facilitates microglial phagocytosis of α-syn, and inhibits its cell-to-cell transmission
- ABL's BBB target is relatively specifically expressed on brain endothelial cells compared to known BBB targets (i.e. TfR)
- ABL301 clearly enters brains and CSF better than anti-α-syn IgG, leading to better efficacy
- ABL301 enters cell line development in Q3 2019, in parallel to characterize its BBB penetration in monkeys and humans (humans: physiologically-based pharmacokinetic (PBPK) modeling)

References

1. Lee H. et al., Extracellular α-synuclein—a novel and crucial factor in Lewy body diseases. Nat. Rev. Neurol. 2014, doi:10.1038/nrneurol.2013.275
2. Rockenstein E. et al., Differential Neuropathological Alterations in Transgenic Mice Expressing a-synuclein From The Platelet-derived Growth Factor and Thy-1 Promoters J. Neurosci. Res. 2002, 68:568–578
3. Stanimirovic D.B. et al., Emerging Technologies for Delivery of Biotherapeutics and Gene Therapy Across the Blood-Brain Barrier. BioDrugs. 2018, https://doi.org/10.1007/s40259-018-0309-y
4. Biogen patent (US8940276B2), Prothena patent (8609820B2), BioArctic patent (WO201104696A1)
5. Choi Y et al., Prion-like Propagation of a-Synuclein Is Regulated by the FcgRIIB-SHP-1/2 Signaling Pathway in Neurons. Cell Reports. 2018, https://doi.org/10.1016/j.celrep.2017.12.009

Acknowledgements

KDDF 범부처신약개발사업단 Korea Drug Development Fund
아주대학교 1973 AJOU UNIVERSITY
서울대학교 SEOUL NATIONAL UNIVERSITY