# Netrin-1 and neurodegenerative diseases: Unraveling therapeutic avenues

Iosif Pediaditakis[1], James McCarthy[2], Ashley J. Mason[1], Ruth Jones[2], Bradley Holub[1], Jane Osbourn[2], Michelle M. Sidor[1]

[1]Alchemab Therapeutics, Waltham, MA, USA, [2]Alchemab Therapeutics, Whittlesford, Cambridge, United Kingdom

NO – PHOTO / RECORD

ALCHEMAB THERAPEUTICS

## Abstract

Netrin-1, a key player in embryonic axonal guidance, has a significant role in promoting adult synaptic plasticity and neuroprotection. Recent studies have reported altered Netrin-1 levels in neurodegenerative diseases such as Parkinson's Disease (PD) and Alzheimer's disease (AD), suggesting its potential role in disease pathogenesis and/or treatment. We explored Netrin-1's role in neurodegeneration via immunodepletion of Netrin-1 in iPSC-derived motor and dopaminergic neuron cultures, αSyn fibril-induced iPSC models of PD, and TDP43 iMotor neuron models of ALS (Q331K variant). Our data indicate that Netrin-1 depletion induces loss of synaptic proteins and leads to cell death. Further experiments, using two-compartmental microfluidic devices, revealed axonal reduction induced by Netrin-1 deprivation, suggesting a link between Netrin-1 levels and neuronal health. Treatment with recombinant Netrin-1 in disease model systems had effects at both the molecular and network level. Netrin-1 treatment increased key synaptic proteins involved in synaptic function and formation and significantly enhanced neuronal functional connectivity and firing behaviour, as indicated by changes in mean neural firing rate and network burst frequency. Overall, these findings suggest that Netrin-1 has the potential to reverse key pathological features associated with PD and ALS, such as cell death and synaptic dysfunction. These findings pave the way for further exploration of the roles of Netrin-1 and its receptors in neural health and disease.

## Netrin-1 depletion induces cell death in neurons

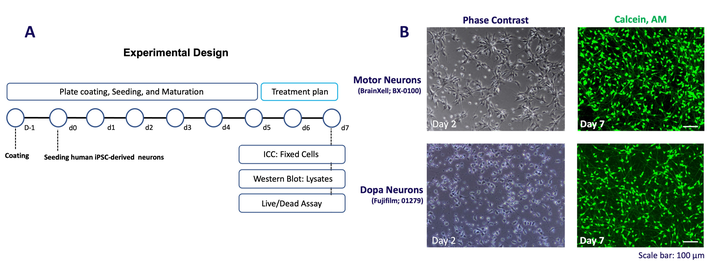


**Figure 1: Adherent growth and neurite outgrowth of human iPSC-derived motor and dopaminergic neurons in culture**. (A) Experimental design: Human iPSC-derived neurons were cultured, and their growth and neurite outgrowth were examined within the first week. (B) Calcein staining (green) illustrates the robust neurite outgrowth and adherence of these neurons during the initial week of culture.

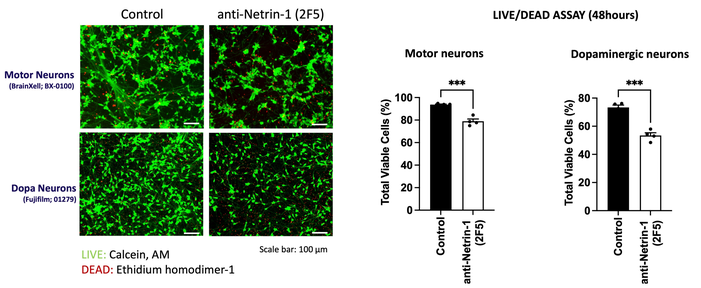


**Figure 2: Impact of Netrin-1 deprivation on neuronal viability after 48 hours of treatment.** On Day 5 of culture, neurons were exposed to 2F5 (10 µg/ml), which is a Netrin-1 monoclonal antibody that neutralizes secreted Netrin-1, or they were exposed to PBS (control). Following a 48-hour incubation, cell viability was assessed using the LIVE/DEAD staining kit, with neurons incubated in calcein AM and ethidium homodimer-1. Images were obtained at ×10 magnification using Fiji/ImageJ. Results are presented as the average live cell percentage relative to total cell count, revealing Netrin-1's potential role in neuronal survival (DIV: days *in vitro*). Statistical significance was determined using one-way ANOVA with analysis conducted on four randomly selected areas per well, ***P<0.001. Error bars represent the mean ± SEM.

## Netrin-1 depletion reduces axonal growth

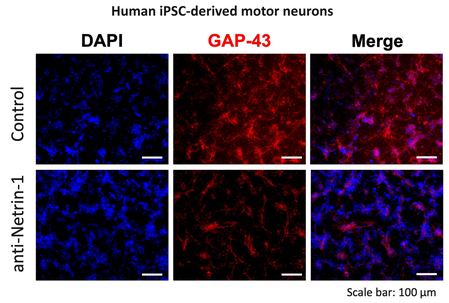


**Figure 3: Netrin-1 depletion reduces the levels of GAP-43, an indicator of neurite elongation and synapse formation**. Immunostaining revealed decreased GAP-43 after Netrin-1 depletion. GAP-43 antibody (ThermoFisher; PIPA579299) incubation, and Alexa Fluor-568 secondary antibody (Abcam, ab175470) were followed by DAPI staining. Images taken with Echo Revolve microscope.

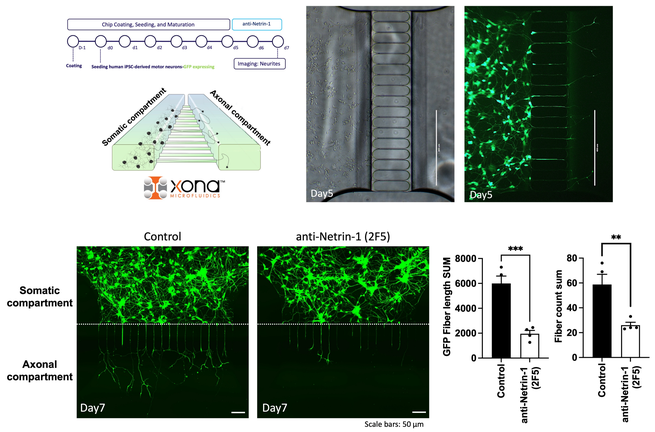


**Figure 4: Netrin-1 deprivation-Induced axonal reduction.** Employing microfluidic devices, we uncovered a reduction in axonal structures attributed to Netrin-1 deprivation. The study utilized eGFP spinal motor neurons cultured on XonaChip SF150X4 "X4" culture units. After 48 hours of incubation, microscopic examination revealed a noticeable decrease in axonal density. This finding underscores the pivotal role of Netrin-1 in maintaining axonal integrity. Statistical analysis is two-sided unpaired t-test (n = 4 independent units), **P < 0.01, ***P < 0.001 to Isotype control group. Error bars represent the mean ± SEM.

## Netrin-1 enhances synaptic plasticity in a hiPSC-derived ALS model

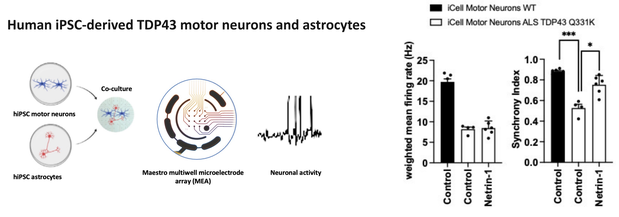


**Figure 5**: **Netrin-1 boosts synchrony and synaptic strength in TDP43 motor neurons**. ALS TDP43 motor neurons display altered connectivity compared to isogenic counterparts. Netrin-1 enhances synchrony without changing firing rates, strengthening specific synaptic connections in ALS TDP43 neurons relative to the control (PBS). Three-way ANOVA (mixed effects model) with post-hoc Tukey for multiple comparisons. n=4-6 wells/treatment group, ***P<0.001, *P<0.05. Error bars represent the mean ± SEM.

## Netrin-1 promotes synaptic function and formation

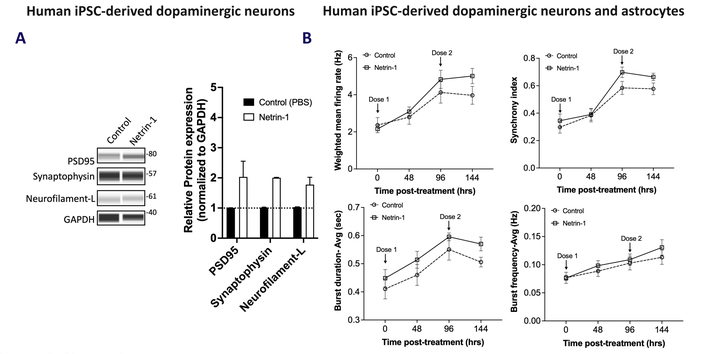


**Figure 6: Netrin-1 elevates synaptic proteins and enhances connectivity in dopaminergic neurons**. (A) Immunoblotting data demonstrates that Netrin-1 treatment leads to a substantial augmentation in synaptic protein levels within dopaminergic neurons, establishing its potential to enhance synaptic function and facilitate the formation of synapses. (B) MEA data reveals that Netrin-1 treatment enhances synchrony and firing rates, strengthening specific synaptic connections in a co-culture system with dopaminergic neurons and astrocytes.

## Neuroprotective role of Netrin-1 in αSyn-induced model

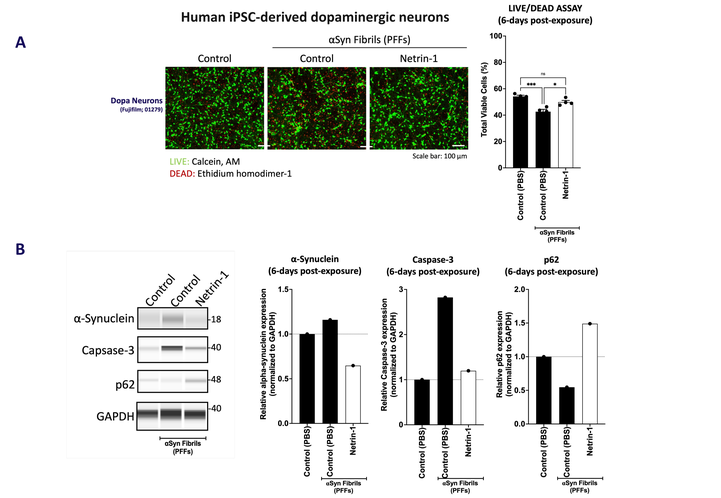


**Figure 7: Netrin-1's neuroprotective effects in αSyn fibril-Induced neuronal loss.** (A) Dopaminergic neurons (DIV 11) were exposed to αSyn fibrils (4 µg/ml) or PBS (control) on Day 5. After 6-days of incubation, cell viability was assessed using a LIVE/DEAD staining kit. Statistical significance was determined by one-way ANOVA (***P<0.001, *P<0.05). Error bars represent mean ± SEM. (B) Immunoblotting revealed that αSyn fibril exposure led to increased αSyn accumulation and cell death. p62 levels decreased with αSyn fibril treatment, suggesting impaired lysosomal degradation and autophagosome buildup. Netrin-1 treatment reduced neuronal loss (lowered Caspase-3) and increased p62 levels compared to the non-treated αSyn fibrils group, highlighting its potential against Parkinson's disease pathology.

## Conclusions

- Deprivation of Netrin-1 resulted in axonal reduction, partially rescued by recombinant Netrin-1, suggesting its potential to support synaptic function and formation.
- Netrin-1 treatment significantly influenced neuronal functional connectivity and altered mean firing rates and network burst frequencies.
- Netrin-1 exhibits promise in mitigating crucial pathological features linked to Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS), including cell death and synaptic dysfunction.

# Human-derived CD33 antibody ATLX-1088: A potent stimulator of microglial pharmacology

Helen Graves[2], Galina Nosovitskaya[2], Carlo Zimarino[2], Han Cai[2], Donna Finch[2], Iosif Pediaditakis[1],Laura Mitchell[2], Amparo Toboso-Navasa[2], Paulina Kolasinska-Zwierz[2], Urszula Gawda[2], Jake Galson[2] , Sanjay Keswani[2], Paul Varley[2], Ralph Minter[2], Jane Osbourn[2], Michelle M. Sidor[1]

[1]Alchemab Therapeutics, Waltham, MA, USA, [2]Alchemab Therapeutics, Whittlesford, Cambridge, United Kingdom

NO – PHOTO / RECORD

ALCHEMAB THERAPEUTICS

## Introduction

At Alchemab, we are harnessing the power of the immune system to find novel therapies for complex diseases. Through target-agnostic screening of immune repertoires from resilient individuals, we identify antibodies associated with resilience. Using this approach, we have identified CD33 as an antigen of interest through screening individuals with dementia risk factors (elevated beta-amyloid, APOE4 risk allele) but no cognitive decline or Tau elevation. Further repertoire mining identified a larger panel of CD33 antibodies which were characterized for their target specificity, CD33 binding affinity, cell surface CD33 binding, CD33-mediated internalization, and functional effects in iPSC-derived microglia. This screening cascade identified a human-derived lead antibody, ATLX-1088, which was found to be a strong inducer of microglia phagocytosis of beta-amyloid. In comparison to other CD33 and TREM2 antibodies, ATLX-1088 was superior in increasing phagocytosis of iPSC-microglia. Interestingly, ATLX-1088 also demonstrated a greatly reduced level of CD33 depletion from the cell surface of human monocytes, which was strikingly different from other known antibodies to CD33. From a development perspective, this antibody shows high levels of monomer, good thermostability, and minimal loss of binding under forced degradation conditions. Overall, these data suggest that ATLX-1088 has high potential as a therapeutic candidate for the reversal of microglial dysfunction in Alzheimer's Disease and potentially other neurodegenerative conditions.

## Alchemab's discovery engine: From patient to target

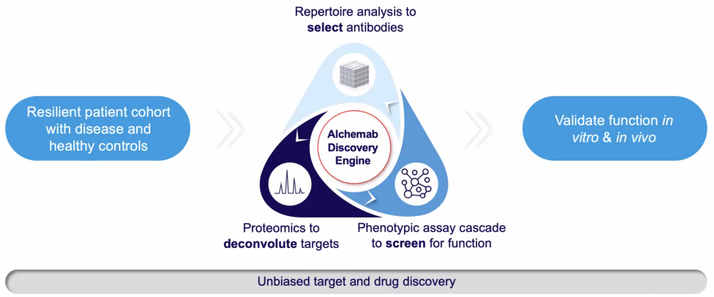


**Figure 1. Alchemab's target discovery engine.** We source patient (both resilient and progressor) and healthy control samples and sequence their B-cell repertoires to uncover convergent antibodies that are unique to the resilient patient population. These antibodies then progress into our discovery workflow, as outlined above.

## Antibody discovered in individuals at risk for Alzheimer's

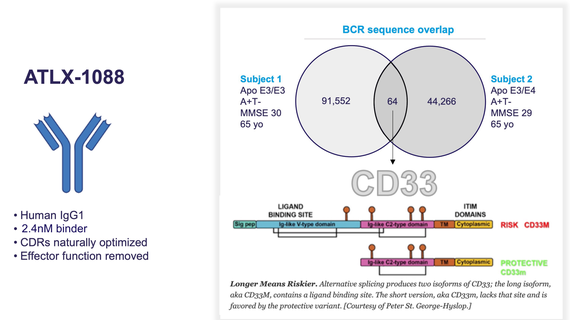


**Figure 2. CD33 antibodies identified from individuals with Alzheimer's disease risk factors.** Two amyloid-positive, tau-negative individuals with preserved cognition showed convergent CD33 expression. Further mining of a 100+ cohort identified CD33 mAbs with inhibitory function, including the lead mAb ATLX-1088. Resilient individuals' antibodies bound CD33 near the ligand site.

## ATLX-1088 significantly enhances microglial phagocytosis

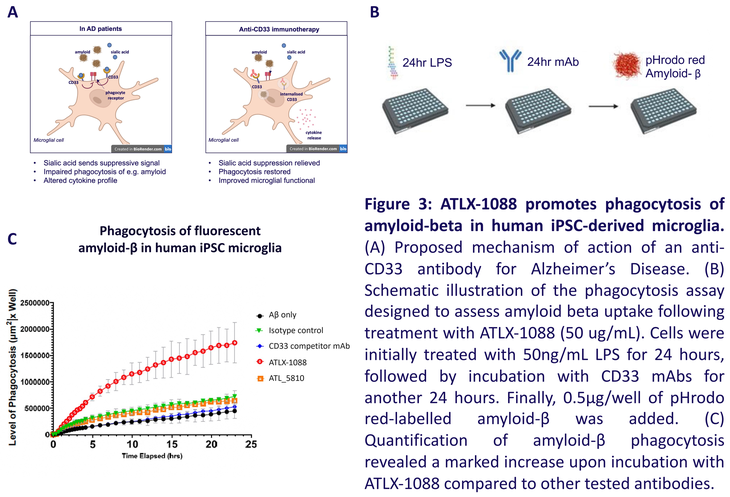


**Figure 3: ATLX-1088 promotes phagocytosis of amyloid-beta in human iPSC-derived microglia.** (A) Proposed mechanism of action of an anti-CD33 antibody for Alzheimer's Disease. (B) Schematic illustration of the phagocytosis assay designed to assess amyloid beta uptake following treatment with ATLX-1088 (50 ug/mL). Cells were initially treated with 50ng/mL LPS for 24 hours, followed by incubation with CD33 mAbs for another 24 hours. Finally, 0.5μg/well of pHrodo red-labelled amyloid-β was added. (C) Quantification of amyloid-β phagocytosis revealed a marked increase upon incubation with ATLX-1088 compared to other tested antibodies.

## ATLX-1088 outperforms clinical stage microglial modulators

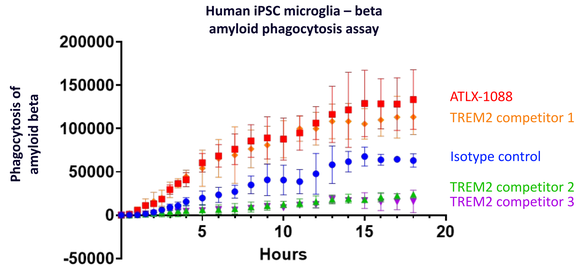


**Figure 4:** iPSC microglial cells were primed with 50ng/mL LPS for 24 hours, followed by incubation with CD33 mAbs for another 24 hours. Finally, 0.5μg/well of pHrodo red-labelled amyloid-β was added in order to visual uptake and quantify phagocytosis. ATLX-1088 exhibited enhanced amyloid-β phagocytosis compared to competitor TREM2 mAbs.

## Lack of internalization delivers benefit to pharmacokinetics

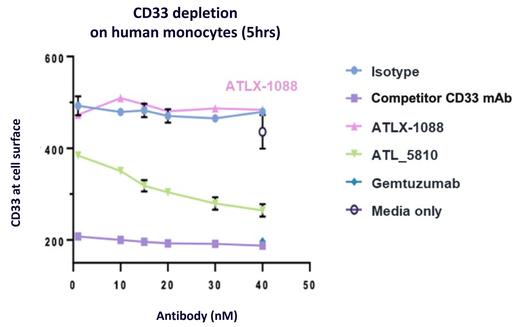


**Figure 5: Preservation of CD33 surface expression with ATLX-1088.** Following a 5-hour treatment with test mAbs, cells were stained with a CD33 C2-domain mAb, HIM3-4, to quantify cell surface levels of CD33. Unlike other CD33 antibodies, there was no loss of CD33 from the cell surface with ATLX-1088 treatment. This has implications and possible benefit for ATLX-1088's PK profile.

## Tri-culture platform: Bridge to a human translational system

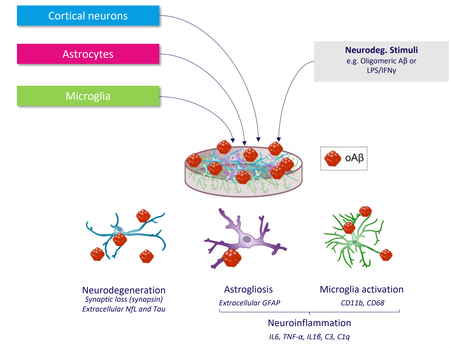


**Figure 6: Schematic representation of a co-culture system for studying CD33 modulation in disease pathology.** The co-culture model incorporates various cell types to facilitate the investigation of inter-cellular dynamics and the impact of CD33 modulation as a potential therapeutic strategy. In contrast to traditional mono-culture approaches, this system has the potential to reveal synergistic effects and complex cellular interactions. Additionally, this model serves as an intermediate step in the translational research continuum, bridging the gap between *in vivo* studies and clinical trials.

## In vivo efficacy: Human microglia in an AD mouse model

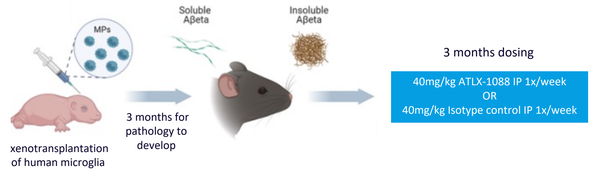


**Figure 7: Study design for engrafted App$^{NL-G-F}$ mice with human microglia transplantation.** The schematic illustrates the unique model of App$^{NL-G-F}$ mice with human microglia transplanted into their brains, offering a novel approach to studying Alzheimer's disease mechanisms and for testing therapeutics, such as ATLX-1088. This model was developed by Prof. Renzo Mancuso from VIB-UAntwerp Center for Molecular Neurology and provides an innovative platform for understanding the interactions between human microglial cells and Alzheimer's disease-associated pathology. The diagram shows the key stages in the transplantation, development, and subsequent monitoring of the engrafted mice.

## Summary

- We have identified ATLX-1088 from an Alzheimer's resilient cohort that targets CD33 – a protein implicated in AD progression and dynamics.
- ATLX-1088 increased phagocytosis of amyloid-β in iPSC–derived microglial cultures and outperformed both a competitor CD33 antibody and clinical-stage microglial modulators.
- Unlike other CD33-targeting therapies, ATLX-1088 did not exhibit an increase in receptor internalization when tested on the cell surface of human monocytes. This lack of internalization may translate to a better pharmacokinetic profile.
- Next steps involve testing ATLX-1088 in translatable in vitro and in vivo model systems.
- Overall, ATLX-1088 has high potential as a therapeutic candidate for the reversal of microglial dysfunction in Alzheimer's Disease with implications that might extend to other neurodegenerative or neuroinflammatory conditions.