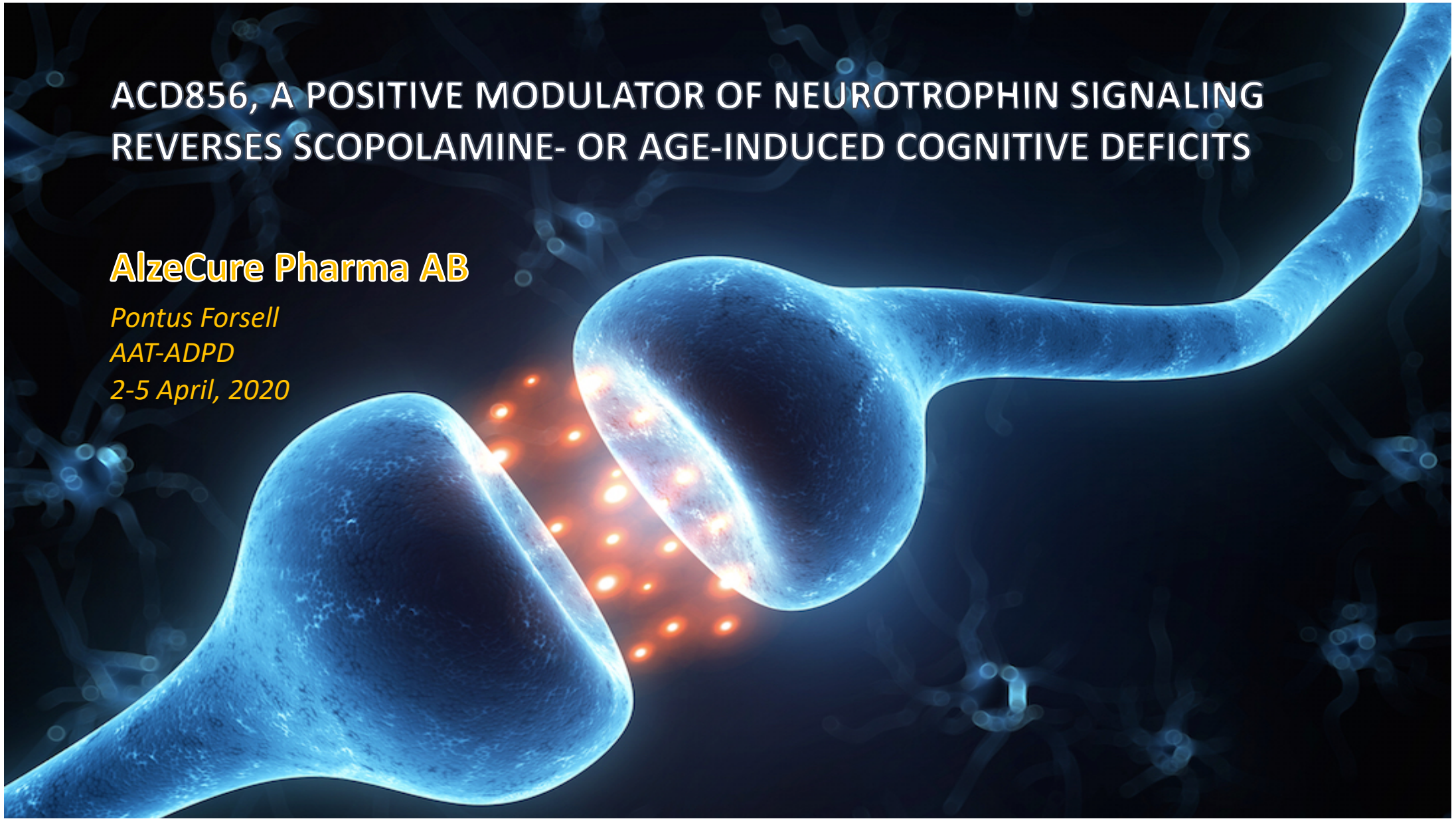

ACD856, A POSITIVE MODULATOR OF NEUROTROPHIN SIGNALING REVERSES SCOPOLAMINE- OR AGE-INDUCED COGNITIVE DEFICITS
AlzeCure Pharma AB
Pontus Forsell
AAT-ADPD
2-5 April, 2020

Disclosures

ADVANCES IN ALZHEIMER'S AND PARKINSON'S THERAPIES
AN AAT-AD/PD™ FOCUS MEETING

2 - 5 APRIL 2020 | VIENNA, AUSTRIA

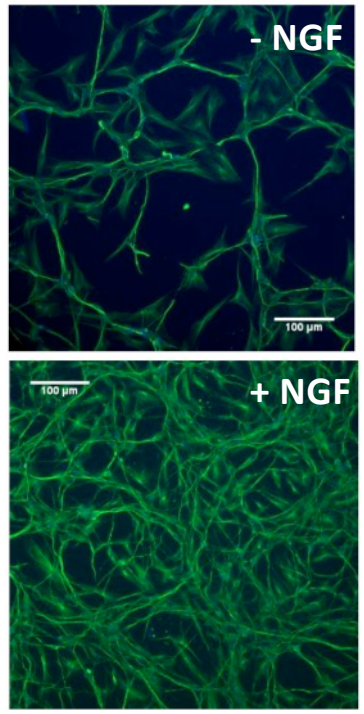
	No, nothing to disclose
X	Yes, please specify:

Company Name	Honoraria/ Expenses	Funded Research	Patent	Stock Options	Ownership Equity	Employee	Affiliation
AlzeCure Foundation	X	X	X				
AlzeCure Pharma AB		X	X		X	X	
Karolinska Institutet							X

Background to NGF & BDNF signaling in AD or PD

- Nerve growth factor (NGF) was discovered in the early 1950s due to its trophic effects on neurons (Levi-Montalcini and Hamburger, 1951)
- Brain-derived neurotrophic factor (BDNF) was initially purified from pig brain (Barde et al., 1982) and shown to promote survival of DRG neurons
- NGF & BDNF signaling are implicated in several neuronal functions, including cholinergic function, hippocampal neurogenesis and synaptic plasticity (LTP)
- Loss of NGF-dependent cholinergic neurons in the basal forebrain and hippocampal atrophy are early hallmarks of Alzheimer's disease and correlates with cognitive decline
- The BDNF-Val66Met polymorphism is associated with cognitive impairment in both AD and PD

Human DRGs



Reduced NGF and/or BDNF-levels might limit the brains ability to withstand pathological injury

BDNF signaling is a genetically validated pathway in AD and PD

BDNF Val66Met heterozygotes

Met allele - 30% reduction in BDNF secretion

Prevalence: 37% of Caucasians, up to 70% of Asians

Familial AD

- Impaired memory performance,
- Reduced hippocampal glucose metabolism
- Increased CSF Tau and p-Tau

Sporadic AD

- Increased rates of decline in memory and hippocampal atrophy

Cognition in PD

- Conflicting reports but Val66Met could be important for cognitive outcome (Altmann, V., 2016)

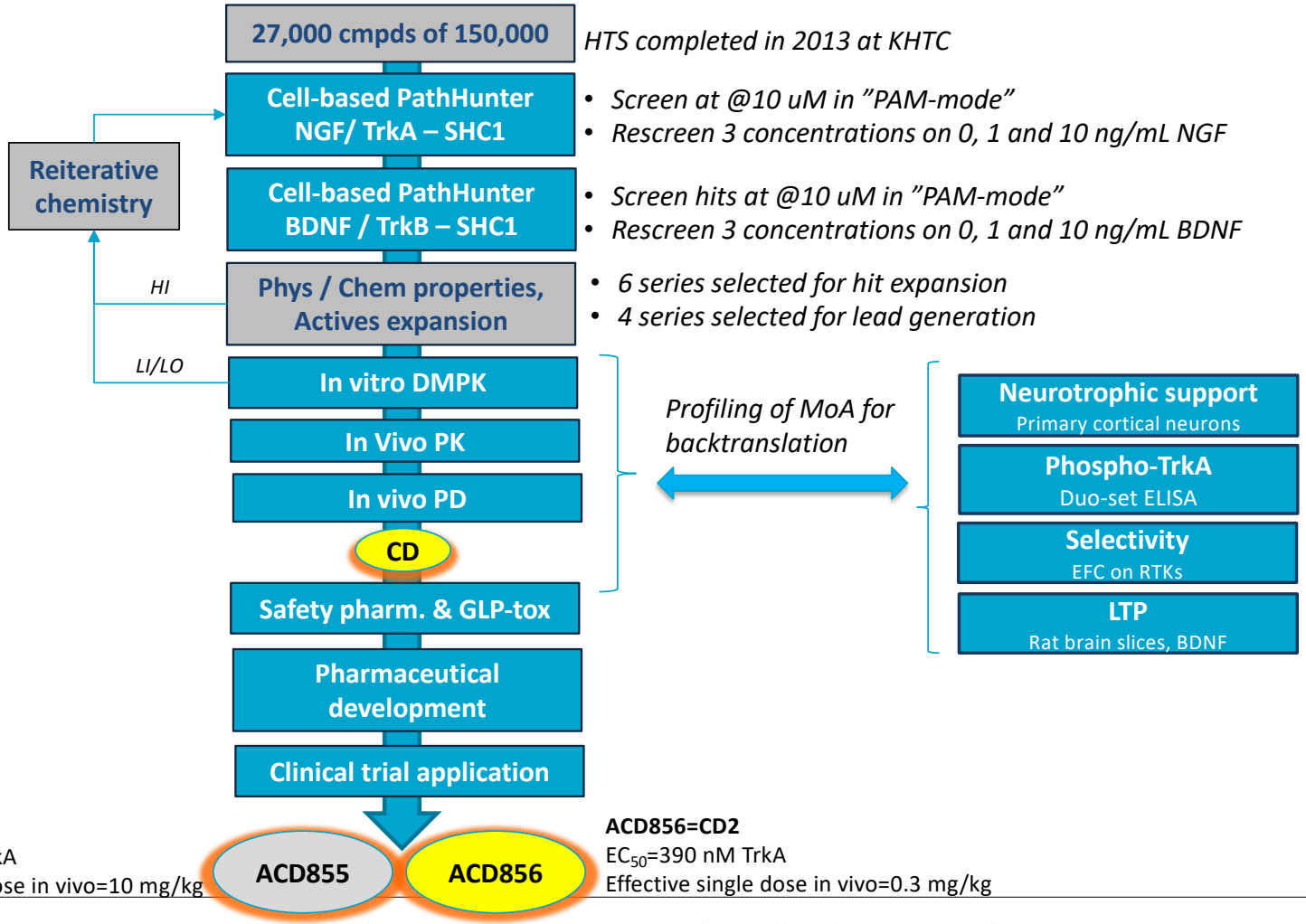
"To date, this is the only genetic factor found to moderate downstream effects of amyloid-β levels in autosomal dominant Alzheimer's disease."
Bateman *et al.*, Brain, 2016 (DIAN, Dominantly Inherited Alzheimer Network)

Positive modulators of TrkA and TrkB signaling could improve neuronal function in AD and PD, thereby functioning as cognitive enhancers

ACD856, study outline

- **Objectives**
  - There are numerous studies demonstrating that neurotrophins such as NGF and BDNF are important for cognitive function. The genetic linkage between BDNF and cognitive dysfunction in AD and PD also strongly support the **development of positive modulators of neurotrophin signaling as novel cognitive enhancers.**
- **Methods**
  - A drug discovery program for identification of positive modulators of neurotrophin signaling was initiated. Compounds were characterized in different *in vitro* assays and in behavioural models in mice. In vivo micro dialysis was performed in the hippocampus of rats.
- **Results**
  - ACD856, and its predecessor ACD855, were identified after the lead optimization program as positive modulators of NGF/TrkA- and BDNF/TrkB-signaling

Discovery of ACD855 and ACD856

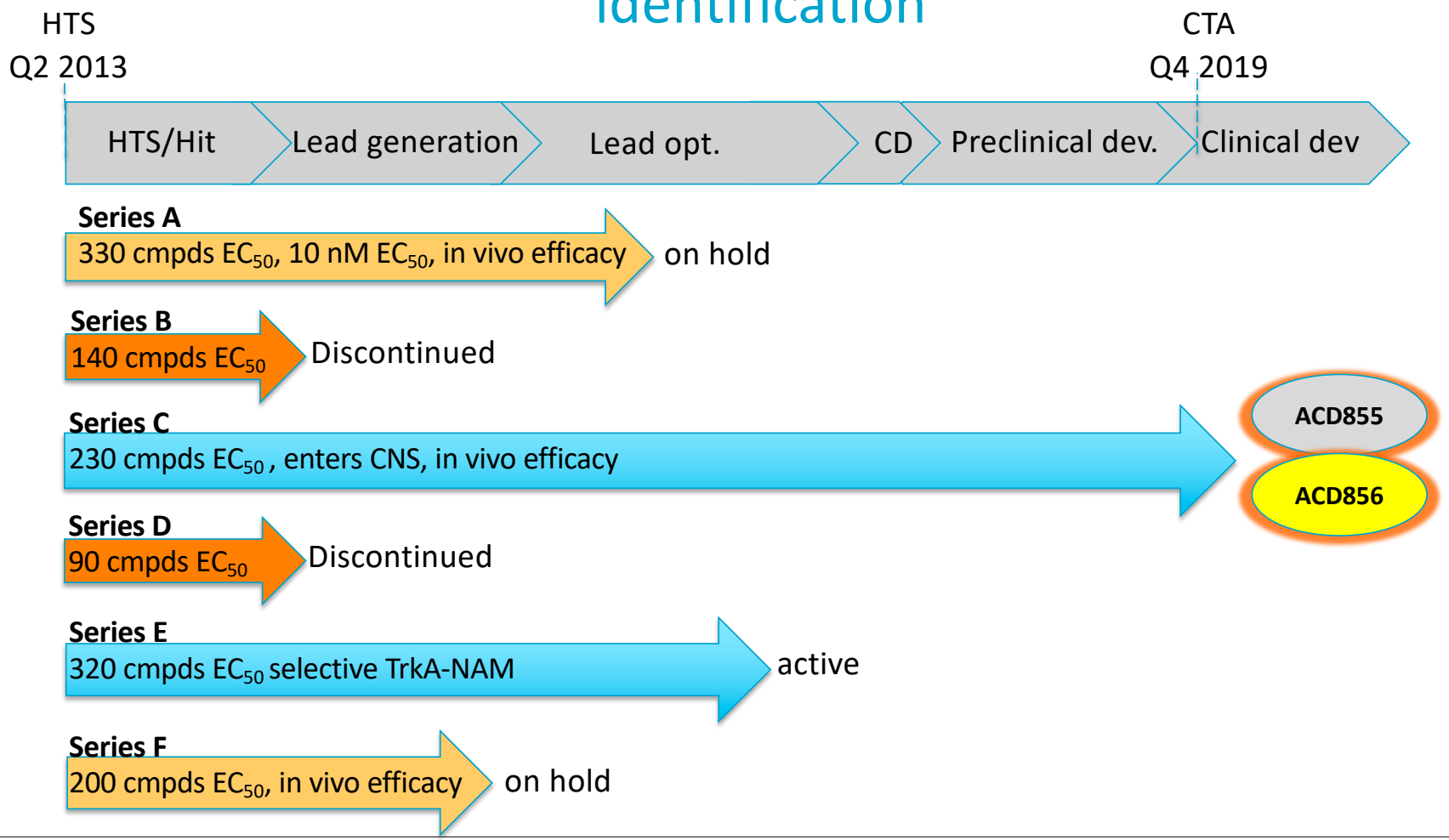


ACD855=CD1
EC_{50}=3270 nM TrkA
Effective single dose in vivo=10 mg/kg

ACD856=CD2
EC_{50}=390 nM TrkA
Effective single dose in vivo=0.3 mg/kg

AlzeCure

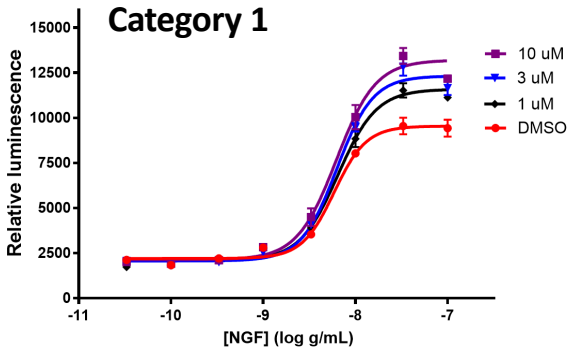
Chemical series originating from hit-identification

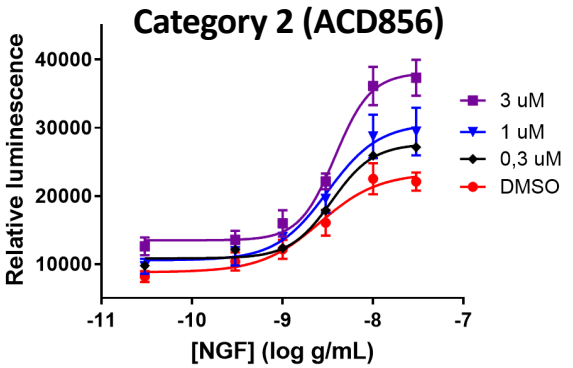


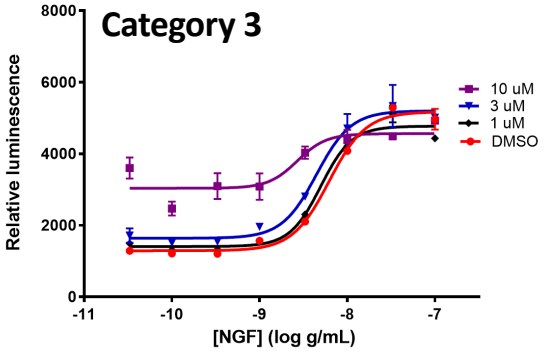
Different mechanisms of action of three selected hits

- **Category 1:** Positive modulators of neurotrophin signaling with no agonistic properties
- **Category 2:** Positive modulators of neurotrophin signaling with low agonistic properties, includes ACD856
- **Category 3:** Partial agonist

Hits with no or low agonistic properties were selected for further optimization



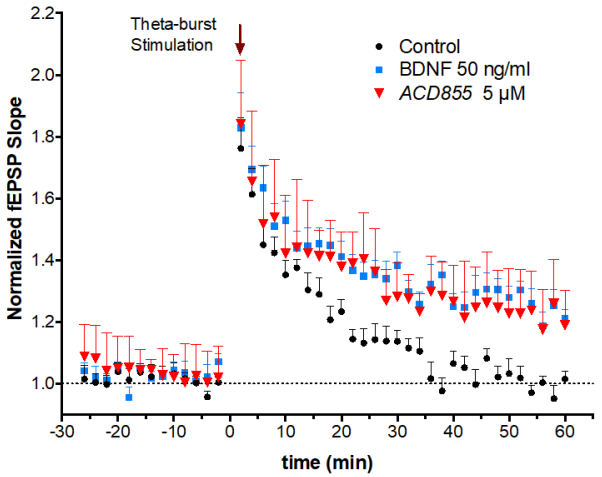




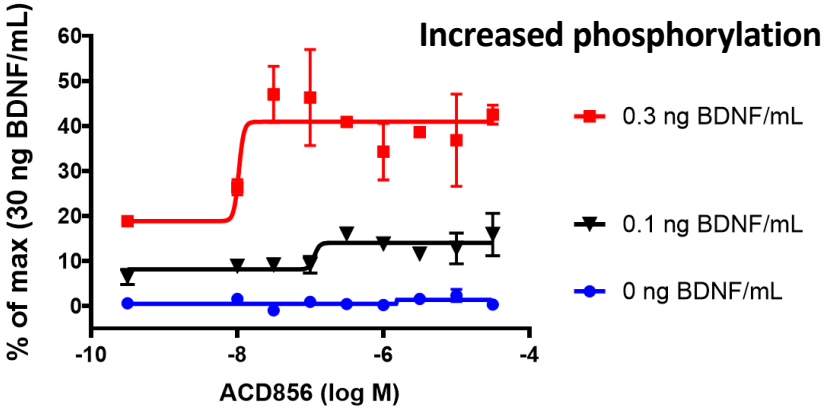
ACD855 and ACD856
In vitro pharmacology

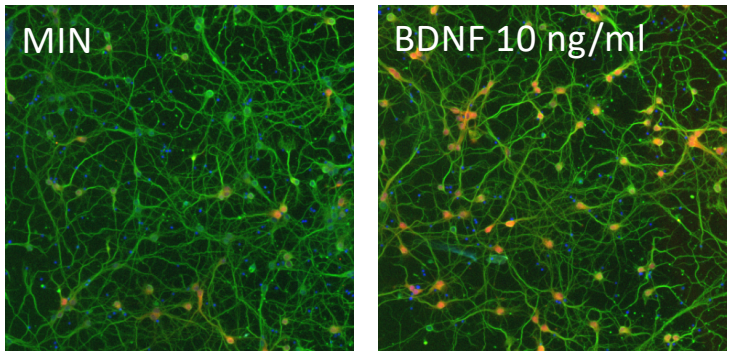
- ACD855 and ACD856 are potent enhancers of human and rodent Trk signaling
  - Increases phosphorylation of Trk-receptors (phospho-Trk ELISA)
  - Increases phosphorylation of ERK1/2 in primary cortical neurons
- Potentiates the induction and maintenance of LTP in rat brain slices in a manner similar to BDNF itself

Potentiation of LTP



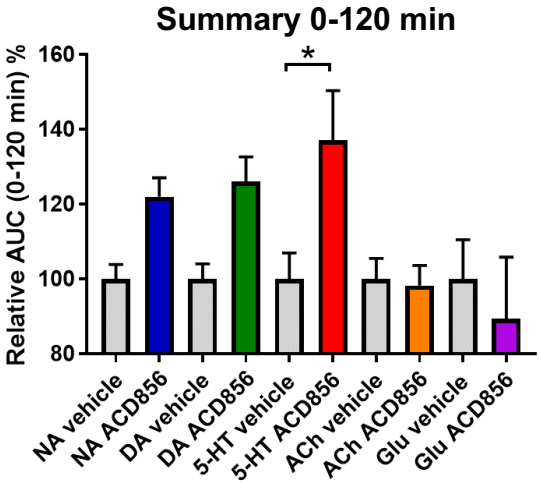
Increased phosphorylation of ERK1/2 in mouse primary cortical neurons

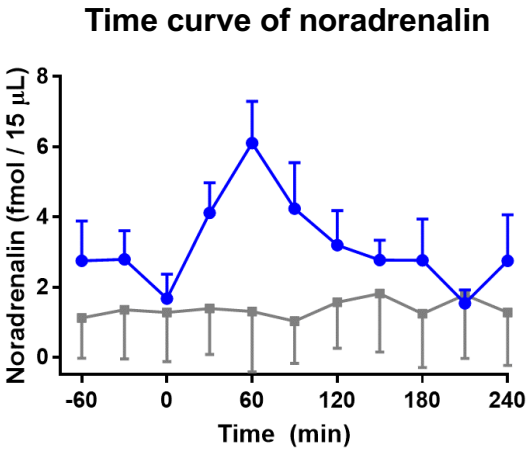


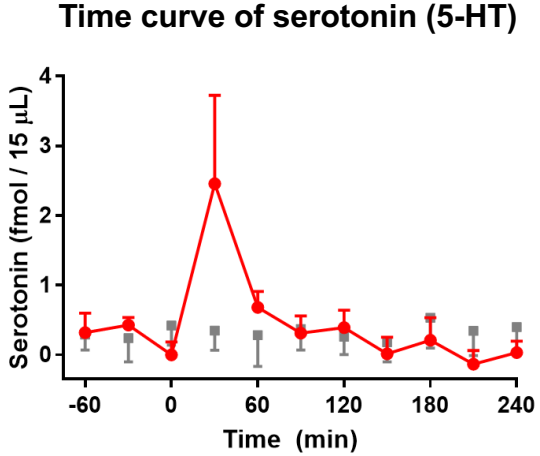


Peripheral administration of ACD856 leads to increased levels of neurotransmitters in the hippocampus

- Microdialysis was performed by placing the dialysis probe in the ventral hippocampus
- ACD856 (10 mg/kg) was administered subcutaneously at t=0 min to freely moving rats
- The levels of noradrenalin (NA), dopamine (DA), serotonin (5-HT), acetylcholine (Ach) and glutamate (Glu) were monitored for 240 minutes and analyzed by LC/MS-MS
- ACD856 significantly increased the levels of serotonin and there was trend to increased levels of noradrenalin and dopamine





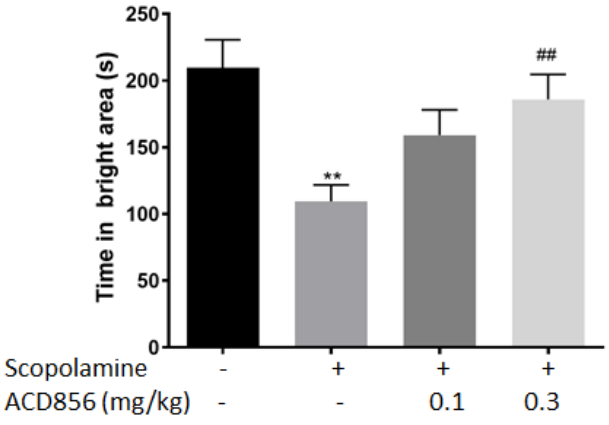


ACD856 increase serotonin levels in the hippocampus
The lack of effect on acetylcholine suggest that ACD856 could be additive to acetylcholinesterase inhibitors

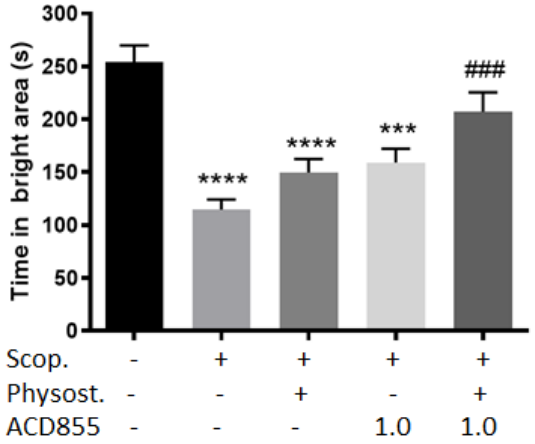
ACD855 and ACD856 attenuates memory impairment in a fear conditioning model in a TrkB-dependent manner

- ACD855 and ACD856 reverses scopolamine or MK-801 induced memory impairment
- No effect of the compounds itself on motor activity or on cognition in young naïve mice
- Lowest dose of ACD856 with significant effect using a single dose is 0.3 mg/kg
- ACD855 is additive to the acetylcholine esterase inhibitor physostigmine
- The effects of the cmpds are blocked by ANA-12, a selective TrkB antagonist

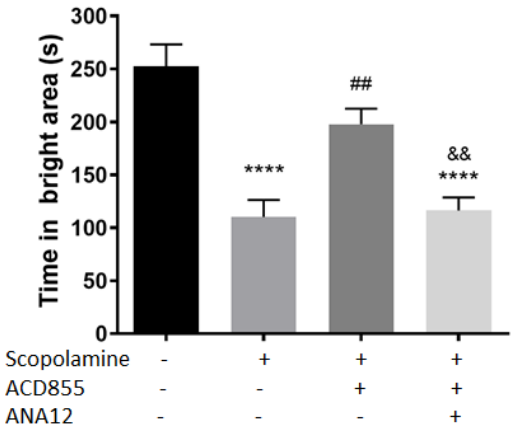
Dose-dependent effects ACD856



ACD855 + physostigmine

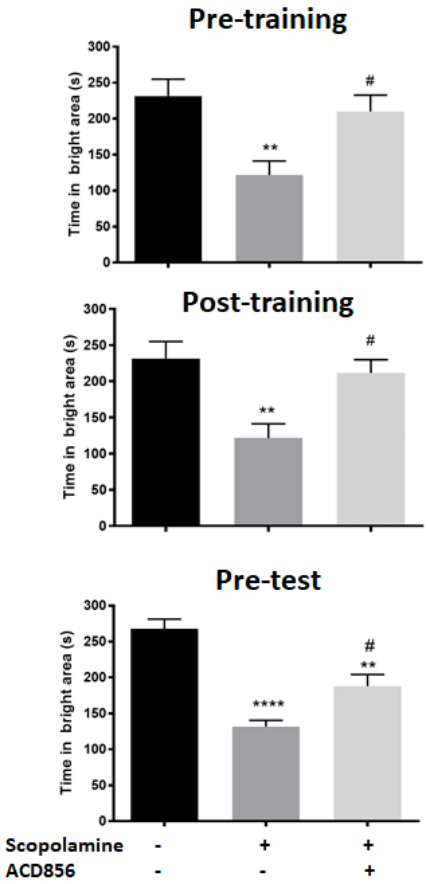


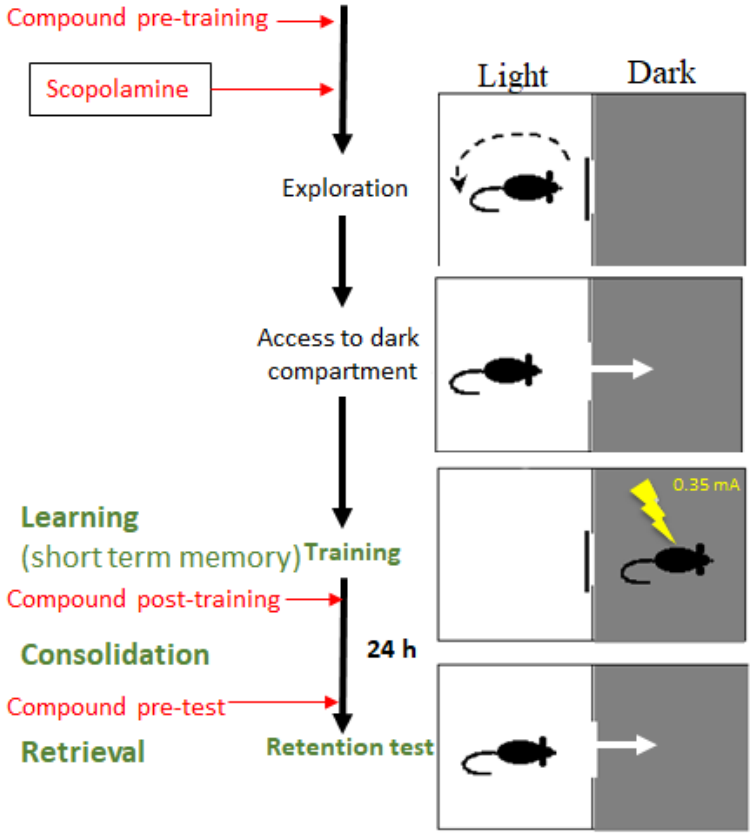
ACD855 + ANA-12



Reversal of induced memory impairment in a TrkB-sensitive manner. The effect is additive to AChEi

ACD856 affects different phases of memory formation

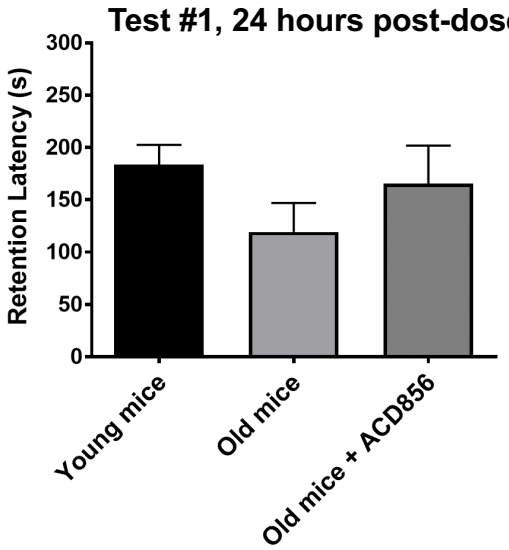


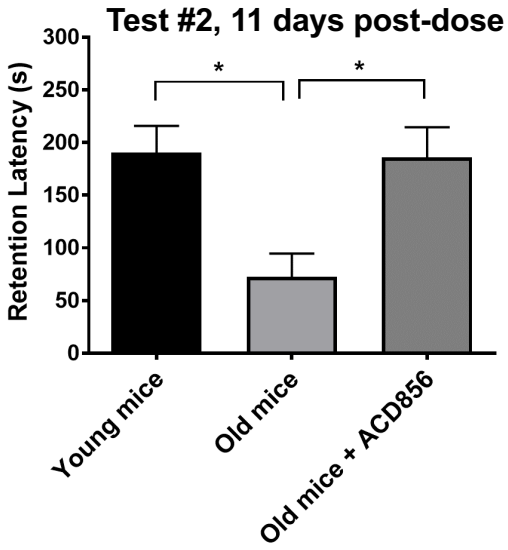


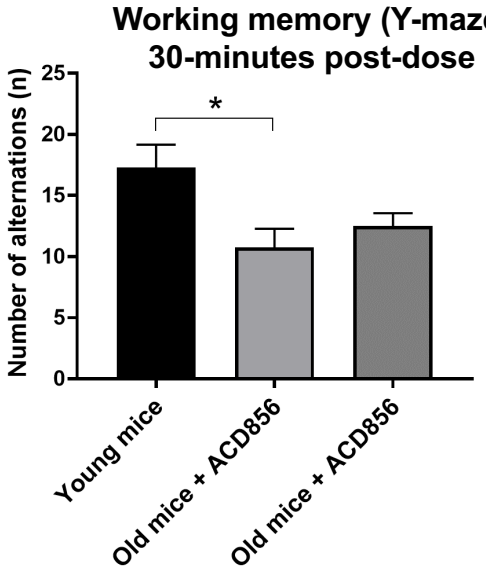
ACD856 improves both acquisition, consolidation and retrieval of long-term memories

ACD856 improves long-term memory in old mice

- Young animals retained a good memory up to 11-days post-training, addressed using a fear conditioning model
- Old animals displayed impaired memory 24 hours after training which was statistically significant 11-days post-training
- ACD856 could fully revert the memory impairment in old animals at day 11
- ACD856 did not show a significant effect on working memory, indicating a hippocampal site of action rather than prefrontal cortical action

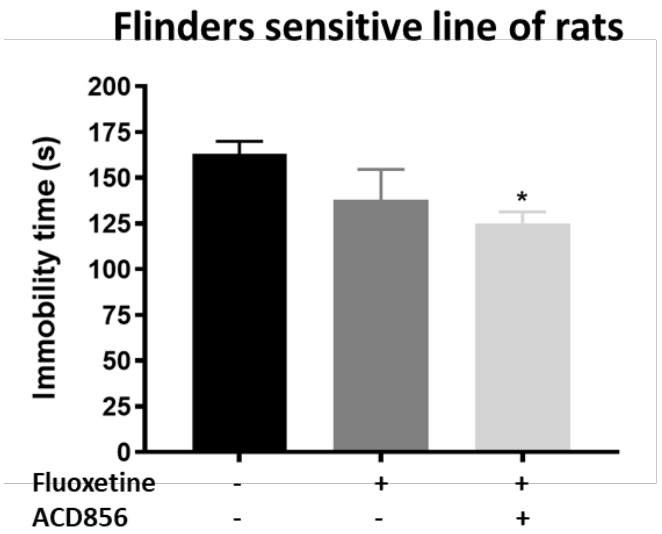


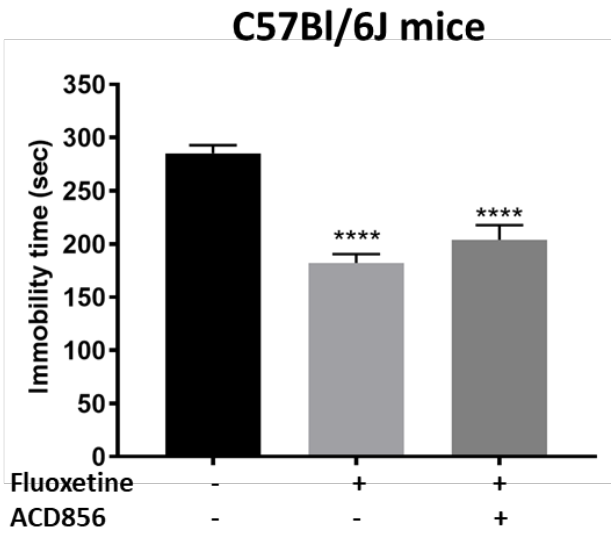




ACD856 is efficacious in a model of depression, forced swim test/Porsolt test

- BDNF signaling has been implicated in depression
- ACD856 was compared to Fluoxetine (SSRI) using the Forced Swim Test
- ACD856 at 1 mg/kg demonstrates similar effect as Fluoxetine 20 mg/kg in both FSL-rats and in mice



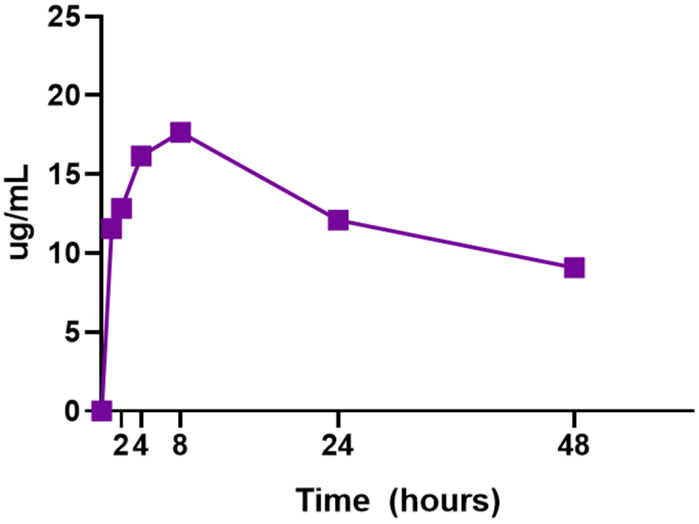


ACD856 displays a range of pharmacodynamic effects suggesting a broad therapeutic use

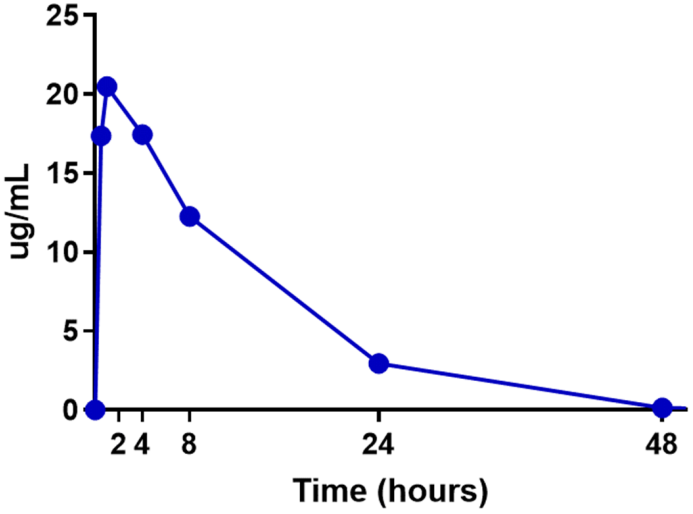
ACD856 shows favorable pharmacokinetic profile in multiple species

- ACD855 demonstrated unexpectedly long-half life in man
- ACD856 shows significantly shorter half-life than ACD855 in mice, rats and mini-pigs

Oral administration of ACD855 in rats



Oral administration of ACD856 in rats



The half-life of ACD856 in animals suggests that dosing once-daily in man can be achievable

ACD856 – DMPK, Safety pharmacology and Toxicology evaluation

- **Pharmacokinetic properties**
  - ACD 856 demonstrates favourable pharmacokinetic properties (mice, rats and minipigs), high bioavailability,, high brain/plasma ratio with high enough exposure in brain
- **Safety pharmacology**
  - ACD856 demonstrate a very benign safety profile with just one hit when assayed against 116 receptors and enzymes
- **Toxicology studies (0, 10, 50 or 300 mg/kg)**
  - No adverse events observed in rats or minipigs during dose-range finding studies and maximum-tolerated dose studies (7-days repeated dosing)
  - The close analogue AC-1122 demonstrated no allodynia or hyperalgesia up to 100 mg/kg using the hot plate model (no results available yet for ACD856)
- **GLP toxicology- and safety pharmacology studies**
  - Formulation allows for high exposure
  - 28-days GLP-toxicology studies and safety pharmacology (CNS, cardiovascular and respiratory) has been initiated
- **A clinical study is ongoing addressing safety and pharmacokinetic profile following intravenous administration**

Plans forward for ACD856

- Clinical phase 1 studies will be initiated at the end of 2020
  - Regular single ascending dose (SAD) study
  - Multiple ascending dose (MAD) study
    - The MAD-study will include an extension/cohort to look for pharmacodynamic effects in healthy volunteers with scopolamine-induced cognitive impairment (Val66Met allele) and in an elderly cohort with very low cognitive impairment

Summary

- ACD856 is more potent *in vitro* and *in vivo* than its predecessor ACD855
- ACd856 is a positive modulator of neurotrophin signaling
- ACD856 significantly increases the levels of serotonin in the hippocampus
- ACD856 attenuates scopolamine-induced cognitive impairment
- ACD856 fully reverses age-induced cognitive impairment to a level comparable to young animals
- ACD856 has good pharmacokinetic properties and bioavailability in animals
- ACD856 is currently in early clinical development

Acknowledgements

AlzeCure

Märta Dahlström, in vitro/ in vivo
Nather Madjid, in vivo
Cristina Parrado, in vitro
Maria Backlund, ADME
Veronica Lindell, in vivo
Johan Sandin, in vivo
Gunnar Nordvall, med chem
Magnus Halldin, DMPK/Safety
Johan Lundkvist, in vitro
Pontus Forsell, in vitro

Karolinska Institutet

Department of Neurobiology, Care Sciences and Society
Prof Bengt Winblad
Assoc. Prof Maria Lindskog
Erika Vazquez-Juarez, Ph.D.
Märta Dahlström, B.Sc.

Karolinska High Throughput Center
Anders Eriksson
Jiangping Liu
Natalia Nekhotiaeva

Alzheimer's Drug Discovery Foundation

Alzheimer fonden

SciLifeLab

VINNOVA Swedens innovation agency

Thank you!

Questions?

You can find us on LinkedIn
Pontus Forsell or Martin Jonsson

You can also e-mail any questions directly to:
Pontus.Forsell@alzecurepharma.com
Martin.Jonsson@alzecurepharma.com