Adrabetadex alone or in combination with arimoclomol and levacetylleucine promotes oligodendrocyte proliferation, synaptic network growth, and myelination in an *in vitro* mouse model of Niemann-Pick disease type C

Poster 2

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BACKGROUND

- Niemann-Pick disease type C (NPC) is a rare neurodegenerative disorder caused by pathologic variants in NPC1 or NPC2, resulting in intracellular cholesterol accumulation within late endosomal and lysosomal compartments and impaired cholesterol availability across the cell.[1,2]
- Accumulated cholesterol and downstream cellular dysfunction drive progressive neurodegeneration and neurological decline.[1-3]
- Adrabetadex is an investigational intrathecal therapy for infantile-onset NPC (I-NPC; neurological onset <6 years of age) and the only therapy designed to directly target accumulated intracellular cholesterol in the CNS, the central pathogenic driver of NPC, by increasing cholesterol redistribution from late endosomal and lysosomal compartments.
- Adrabetadex is also the only therapy shown to improve survival in both early and late I-NPC.[4]
- Other therapies for NPC including arimoclomol and N-acetyl-L-leucine (NALL; levacetylleucine), have distinct mechanisms and may address complementary aspects of the disease biology.[5-8]
- Together, these observations support evaluation of a treatment paradigm in which adrabetadex serves as foundational therapy targeting the causal biology of NPC, with complementary therapies layered to address additional mechanisms and clinical needs.

OBJECTIVES

To evaluate a) the binding interactions between adrabetadex, arimoclomol, and NALL and b) the *in vitro* effects of adrabetadex, arimoclomol, and NALL monotherapy, and adrabetadex in combination with arimoclomol or NALL on oligodendrocyte (OL) proliferation, synaptic network growth, and myelination of mature neurons.

METHODS

- Binding interactions between adrabetadex, arimoclomol, and NALL were evaluated using molecular docking, molecular dynamics simulations, and isothermal titration calorimetry (ITC).
- In an *in vitro* myelination model, primary co-cultures of embryonic *Npc1-/-* mouse neurons and glia, including OLs, were treated continuously from days 12-21 of culture (Figure 1).
- At day 21, immunostaining of myelin-associated glycoprotein-positive (MAG+) OLs and neurofilament-positive (NF+) neurites was used to measure neurite growth from mature neurons and quantify synaptic network (Figure 2A), OL differentiation (Figure 2B), and changes in myelination (length of NF+ neurites wrapped by MAG+ OLs, Figure 2C) (Figure 1).

Monotherapy

- To evaluate individual effects, the neuron-glia co-cultures were treated with each compound separately (Figure 2, single agents). Each compound was tested at 8 different concentrations spanning physiologically relevant, pharmacologically active ranges for *Npc1-/-* cellular systems. Clemastine (250 nM) and culture medium were used as positive and untreated controls, respectively. All activities were normalized to untreated control. Results are expressed as a percentage of the control condition as mean ± SEM (n = 4-6). Monotherapy data were analyzed by One-way ANOVA followed by Fisher's LSD test (significance = $p < 0.05$) and by nonlinear regression for dose-response with a Hill slope of 1, with goodness of fit verified by R2. Non-linear regression was used to calculate EC50 values (data points excluded from the calculation are indicated by open symbols; Table 2)

Combination therapy

- Co-cultures were treated with vehicle control or with concentrations corresponding to the lowest inactive dose, EC25, and EC50 values observed in monotherapy for OL differentiation, an endpoint for which all 3 compounds showed activity. Combination therapy data were analyzed by One-way ANOVA followed by Fisher's LSD test (significance = $p < 0.05$) to compare all combinations to the control condition and by multiple linear regression. Results are expressed as a percentage of the control condition, presented as mean ± SEM (n = 5-6) (Figure 2).

RESULTS: Adrabetadex demonstrated the greatest potency across endpoints, while complementary therapies produced additive effects at substantially lower concentrations

Drug-drug interaction

- Molecular docking and molecular dynamics simulations predicted no stable binding of arimoclomol or NALL within the guest cavity of adrabetadex (Table 1).
  - Adrabetadex showed very weak or no binding affinity to arimoclomol and NALL in direct binding measurement.
- Consistent with these findings, ITC demonstrated no detectable interaction under conditions in which adrabetadex was bound to cholesterol.

In silico assessment of binding affinity of adrabetadex to arimoclomol and NALL

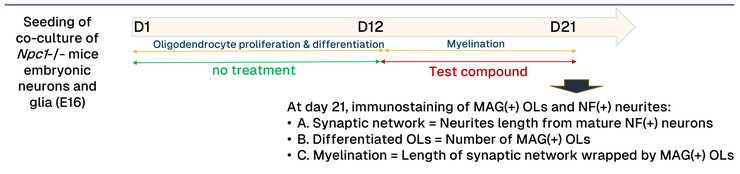
- Molecular dynamics simulations assess pose stability, which measures whether the ligand remains in the adrabetadex cavity over 10 ns. Cholesterol complexes were fully stable; arimoclomol was markedly less so (Table 1).
- APR (attach-pull-release) free energy results indicate that adrabetadex binds cholesterol ~3 orders of magnitude more strongly than NALL and ~3-4 orders more strongly than arimoclomol.
- Convergence across docking, molecular dynamics, and free energy methods reinforces the robustness of these binding affinity predictions.

Table 1. *In silico* assessment of binding affinity of adrabetadex to arimoclomol and NALL

Host	Guest	Docking (kcal/mol)	MD Simulation	Free Energy Simulation[a] (kcal/mol)	Binding Affinity Simulation (ITC)
Adrabetadex	Cholesterol	-4.8 ± 0.4	10/10 Stable	-9.0 ± 0.6	1353 ± 430 µM
	Arimoclomol	-3.8 ± 0.1	4/10 Stable	-3.7 ± 0.6	ND
	NALL	-2.7 ± 0.6	9/10 Stable	-4.9 ± 0.6	ND

Docking and free energy simulations estimate binding free energy (ΔG), which relates directly to affinity (ΔG = RT ln Kd); more negative values indicate stronger binding. ND, no binding detected.
[a]Due to cost, free energy simulation is performed to only one bound pose.

Figure 1. Study Design



Monotherapy

- At concentrations between 50 nM and 5 µM, adrabetadex as a single agent significantly increased synaptic network growth (Figure 2A), the number of early differentiated MAG+ OLs (Figure 2B), and induced early myelination between 5 nM and 5 µM (Figure 2C)
- Arimoclomol and NALL alone significantly increased the number of early MAG+ OLs at the 3 highest concentrations tested (Figure 2B)
- Arimoclomol induced early myelination at 1 µM and 10 µM (Figure 2C); however, no significant effect was observed on synaptic network growth (Figure 2A)
- NALL induced early myelination (Figure 2C) and enhanced synaptic network growth at 500 µM only (Figure 2A)
- Across all endpoints, adrabetadex demonstrated higher potency (Table 2)

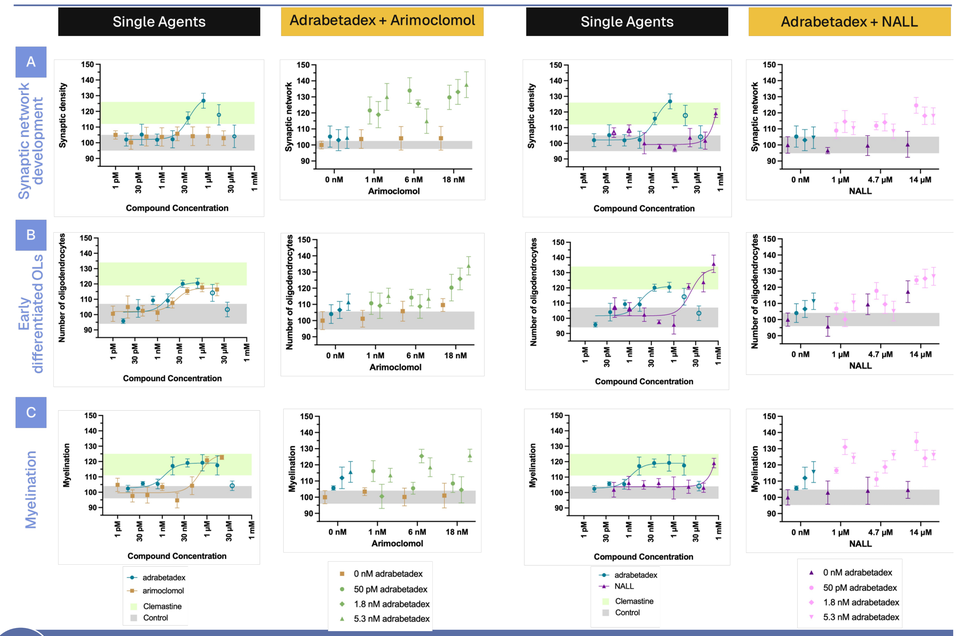
Table 2. EC_{50} values for the tested compounds across evaluated endpoints

Assay	Adrabetadex	Arimoclomol	NALL
Early differentiated OLs	5.3 nM	18 nM	14 µM
Synaptic network	58 nM	–	384 µM
Early myelination	1.4 nM	300 nM	288 µM

Combination therapy

- Multiple linear regression analyses revealed that:
  - Combinations of adrabetadex with arimoclomol demonstrated additive effects in enhancing early OL differentiation and a significant interaction in increasing synaptic network ($p = 0.003$), indicative of synergy. Combinations of adrabetadex with NALL demonstrated additive effects on early myelination.
- One-way ANOVA analyses showed that:
  - All combinations of adrabetadex with arimoclomol at its EC50 (18 nM; Table 2) significantly increased the growth of the synaptic network and the number of MAG+ OLs (Figure 2A and 2B).
  - Several dose combinations of adrabetadex with arimoclomol, including concentrations that were inactive as adrabetadex or arimoclomol monotherapy, increased early myelination (Figure 2C).
  - Similarly, all dose combinations of adrabetadex with NALL at its EC_{50} (14 µM; Table 2) significantly increased the synaptic network, the number of MAG+ early differentiated OLs, and early myelination (Figure 2).

Figure 2. Effect of combination therapy across endpoints



CONCLUSIONS

- Adrabetadex demonstrated greater potency across synaptic network growth, oligodendrocyte maturation, and myelination, supporting its role as a foundational therapy targeting the central pathogenic biology of NPC.
- Combination of adrabetadex with arimoclomol or NALL achieved comparable effects at substantially lower concentrations than monotherapy, supporting mechanistic compatibility between adrabetadex and therapies with complementary mechanisms.
- Functional measures, including synaptic network activity, OL number, and axonal myelination, were preserved or improved with co-dosing.
- No evidence of drug-drug interaction was observed, supporting evaluation of complementary therapies in addition to adrabetadex treatment.
- Together, these findings support a treatment paradigm in which adrabetadex serves as foundational therapy and complementary agents may be layered to address additional aspects of disease biology in I-NPC.

References

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