Novel CD71 Centyrin - siRNA therapeutic targeting the CD40 pathway for autoimmune disorders

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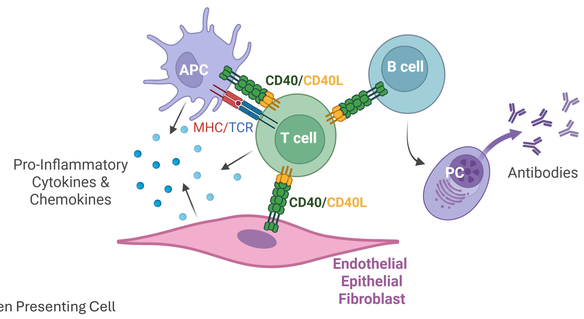
INTRODUCTION

- **CD40** is a transmembrane protein member of the TNF-receptor superfamily, **expressed** on the surface of **myeloid cells**, **B cells**, and many **non-immune cells** (ex: endothelial, epithelial, muscle, fibroblast, microglia and tumor cells).
- **Interactions with CD40L** (CD154) activates the CD40 cascade signaling that are **key for cell homeostasis, activation and function**.
- The **CD40/CD40L pathway is dysregulated in multiple autoimmune disorders** and has been a therapeutic target for over 20 years with clinical validated POC.
- Most of the **early approaches** targeting this pathway encountered **undesired side effects** such as thrombosis, unintended agonism, immune complex deposition and high dose requirements.
- Here we describe the design and use of a **novel CD71 Centyrin – CD40 siRNA conjugate for the treatment of autoimmune disorders.**



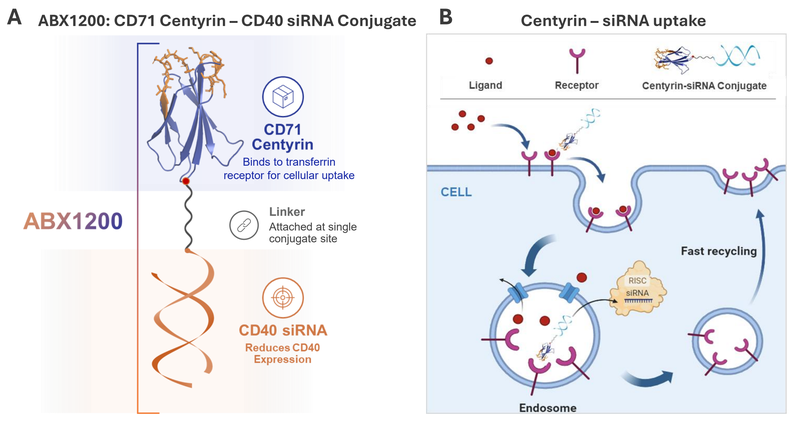
APC = Antigen Presenting Cell
PC = Plasma Cell
MHC= Major Histocompatibility Complex
TCR = T Cell Receptor

ARO'S TECHNOLOGY

A. Aro's ABX1200 conjugate is composed of 3 units:

1. **Centyrin** protein that binds CD71 (transferrin receptor) for cellular uptake.
2. Non-cleavable **linker** for stable siRNA conjugation.
3. CD40-targeting small interference RNA (**siRNA**).

B. Centyrin-siRNA conjugates use the natural receptor-mediated endocytosis for drug uptake. Once in the endosome the siRNA is released into the cytosol where it binds the RISC complex for specific degradation of the targeted mRNA.



Potential advantages of Centyrin - siRNA approach for targeting the CD40/CD40L pathway

- **Targets CD40 mRNA expression** – upstream of CD40 surface receptor.
- **No CD40 or CD40L receptor engagement** – avoids unintended activation.
- **Eliminates potential of immune complexes** – reduced toxicity.
- **Preserves humoral immunity**. *

* Mild to no conjugate activity has been demonstrated so far in B cells with our CD40-siRNA conjugates

DATA

Figure 1. CD71 Centyrin - CD40 siRNA conjugates effectively reduce CD40 mRNA & protein in human dendritic cells *in vitro* with impact on cytokines

(**A**) Human primary monocytes were differentiated into dendritic cells (DCs) by incubation with 100 ng/mL of GM-CSF + 50 ng/mL of IL-4 for 5 days. DCs were then treated with different concentrations of Centyrin - siRNA conjugates in the presence of 100 ng/mL of IFNg for 2-11 days. (**B**) CD40 mRNA expression was analyzed by RT-qPCR, (**C**) CD40 protein was analyzed by flow cytometry, and (**D**) cytokines after 24 h of stimulation with 1 ug/mL of mega-CD40L were assessed by MSD. Mean +/- SD are displayed, *p<0.05 One-way ANOVA.

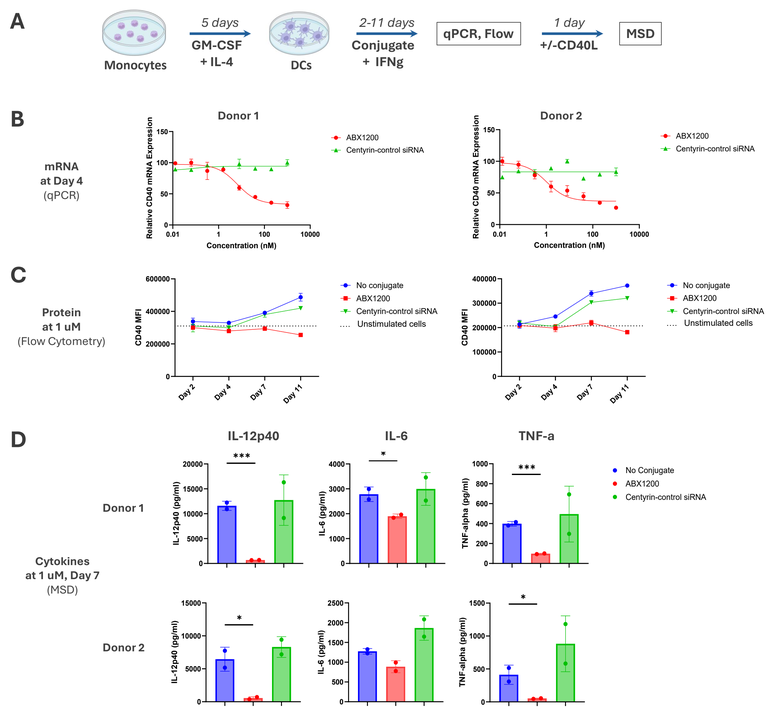


Figure 2. CD71 Centyrin - CD40 siRNA conjugates are active in a CD40 agonist mouse model

(**A**) CD-1 mice were treated with 2 doses of 30 mg/kg of CD40 siRNA conjugates at 3, 6 or 12 days prior to termination, and exposed to CD40 agonist at Day 9. (**B**) Spleen CD11b+ myeloid cells (top) and CD19+ B cells (bottom) were isolated using magnetic sorting. The presence of antisense siRNA was detected by Stem Loop-PCR (left), CD40 mRNA expression was analyzed by RT-qPCR (middle), and CD40 protein was analyzed by flow cytometry (right). (**C**) Systemic cytokines were analyzed by MSD in the serum collected 24 h after CD40 agonist challenge. Mean +/- SD are displayed, *p<0.05 One-way. ANOVA. ULOQ= upper limit of quantification.

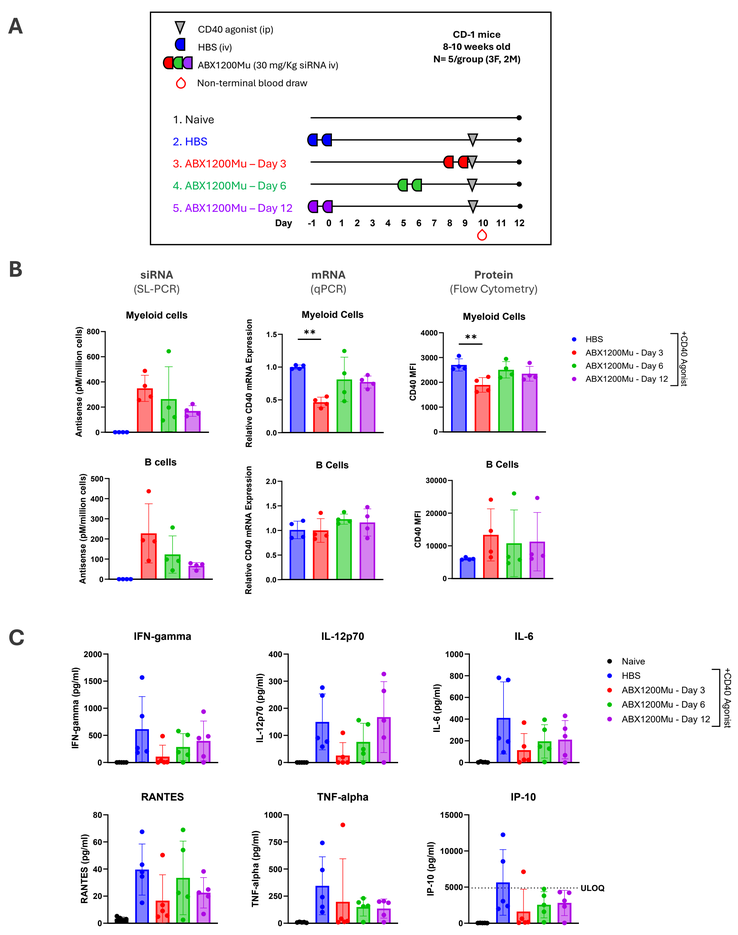
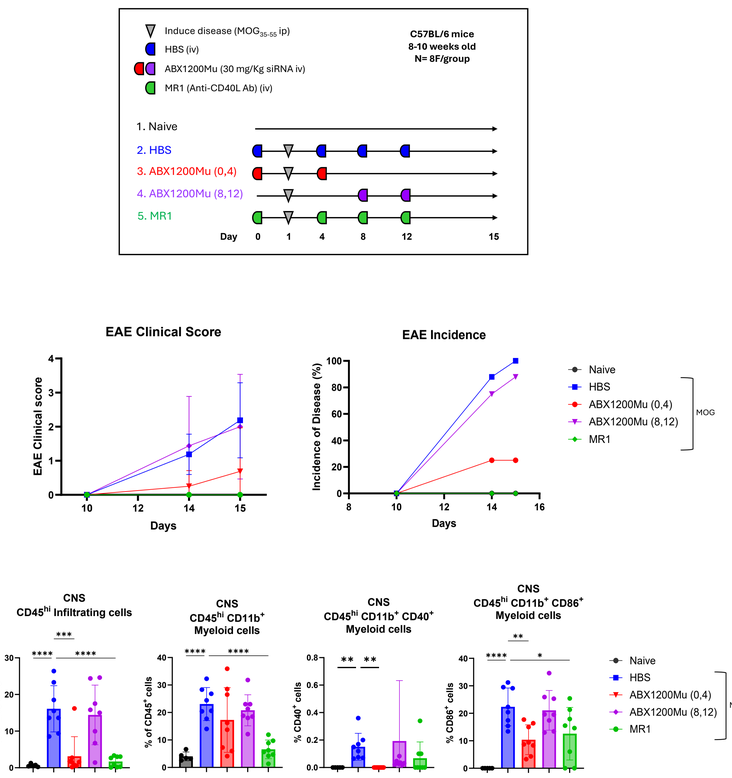


Figure 3. CD71 Centyrin - CD40 siRNA conjugates decrease disease scores and reduce the infiltration, activation and proliferation of myeloid cells in the CNS of mice with Experimental Autoimmune Encephalitis

Experimental Autoimmune Encephalitis (EAE) is a mouse model of Multiple Sclerosis that recapitulates many aspect of the disease, including axonal demyelination, immune cell activation, lymphocyte infiltration into the central nervous system (CNS), secretion of inflammatory cytokines and autoantibodies.

(**A**) C57BL/6 mice were immunized with MOG_{35-55} peptide to induce EAE. 2 doses of 30 mg/kg of CD40-siRNA conjugates were given at days 0 and 4, or 8 and 12. Anti-CD40L antibody (MR1) was given as control treatment. (**B**) Disease scores were recorded until peak of disease (day 15). (**C, D**) On day 15 mice were euthanized, and the spinal cord was processed for flow cytometry analysis of immune cells and microglia. (**E**) Anti-MOG antibodies were analyzed in a separate study in serum collected from diseased animals at Day 21 by ELISA. Mean +/- SD are displayed, *p<0.05 One-way ANOVA.



CONCLUSIONS

CD71 Centyrin - CD40 siRNA conjugates:

- Are **efficiently delivered to immune cells** *in vitro* and *in vivo*.
- **Reduce CD40 mRNA and protein expression** in human and mouse myeloid cells.
- Dramatically **reduce proinflammatory cytokines** *in vitro* and *in vivo*.
- **Ameliorate clinical disease and immune cell activation** in a EAE mouse model of multiple sclerosis.
- Demonstrate **minimal effect on antibody production** in several models, suggesting that efficacy can be achieved while preserving humoral immunity.*

Our data demonstrate that CD71 Centyrin - CD40 siRNA conjugates effectively suppress inflammation in animal disease models as well as primary human myeloid cells; demonstrating exciting potential for continued development as a novel treatment for autoimmune diseases.