

Blocking Nogo to Promote Neuronal Regeneration

PI: Melanie Cocco

Co-PI: Oswald Steward

Background

Since the identification of the myelin protein Nogo-A (RTN-4) as an axonal growth inhibitor, Nogo has been identified as a target in a variety of diseases and conditions specific to the CNS. Nogo antibodies are currently in clinical trials for spinal cord injury, stroke, ALS and have been considered for the treatment of MS. Here, we explore an alternative approach to block Nogo using DNA aptamers that target the Nogo surface responsible for limiting axonal regeneration. Aptamer therapy has several advantages including low cost and long-term chemical stability, and aptamers can be modified to cross the blood brain barrier. The proposed project will develop Nogo-blocking aptamers suitable for therapy (Cocco laboratory) and test in SCI models in vitro (Steward laboratory). The Cocco lab determined the structure of Nogo-66 in a membrane environment using NMR spectroscopy. With no pocket or crevices, the convex surface of Nogo would be difficult to target using small molecules; however, grooves that naturally form in nucleic acid aptamers could efficiently recognize the Nogo helix (**Figure 1**). The surface helix of Nogo-66 contains two Lys and two Arg side chains with no negative charges; this is an ideal target for anionic DNA aptamers.

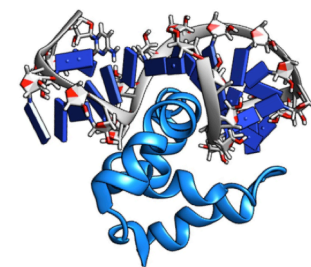


Figure 1. Model of a small 27-nt ssDNA aptamer bound to Nogo-66. This model demonstrates how an aptamer could recognize and block the surface of Nogo responsible for signaling to prevent axonal growth.

Aim 1: Design and Engineering of Aptamers (Cocco). Our preliminary data show that small DNA aptamers selected to bind Nogo-66 promote neurite growth in vitro. We will further engineer and test lead aptamers by *design to increase stability* for both shelf life and to limit nuclease digestion.

Aim 2: Assessment of Biodistribution, Potential Toxicity and Recovery of Neurological Function (Steward). Spinal cord injury models have been the primary test for *in vivo* efficacy of manipulations of Nogo. In spinal cord injury, a plausible therapeutic scenario will involve intrathecal delivery after a spinal cord injury. Accordingly, here we will assess aptamer biodistribution, toxicity and function.

Research Plan

Aim 1: Aptamer screening allows for a large library of structural diversity to be tested for protein binding at the same time. The Cocco lab selected for Nogo binding using a library of ssDNA aptamers with a small variable length of 44 nucleotides (nt). Preliminary data on the best binding aptamers shows that several are functional in blocking Nogo function and, consequently, promoting neuronal growth (**Figure 2**). We currently have a DNA oligo library with a starting sequence diversity of $\sim 10^{22}$; some of these sequences will form helices, loops, and extensive tertiary structures, creating a library of potential recognition surfaces. We envision several routes to improve upon the design of the initial aptamer library tested. We will test a new library of DNA aptamers with an increased length in the variable region, up to 100 nt, to enhance specificity by increasing surface contact area. **Aim 2:** In spinal cord injury, a plausible and likely therapeutic scenario will involve intrathecal delivery after a spinal cord injury. Accordingly, here we will assess biodistribution with intrathecal delivery 1 week post injury. Rats (n=10 per group) will receive thoracic contusion injuries, hindlimb motor function will be assessed using the BBB Scale 2, 5, and 7 days, and then rats will be implanted with intrathecal catheters connected to Alzet minipumps to deliver aptamers. One and 3 days later, locomotor function will be tested by BBB. It should be noted that this is also an initial test for toxicity of the aptamers in the context of a spinal cord injury.

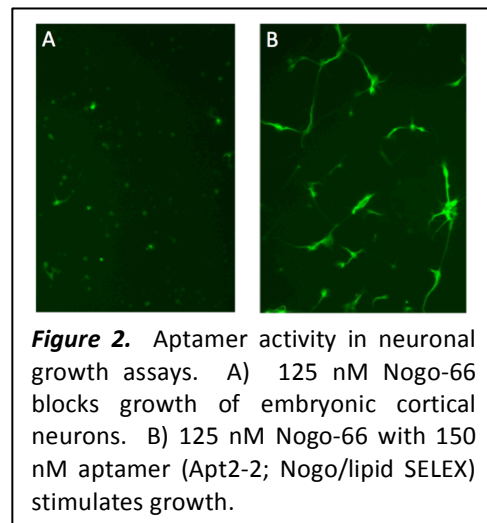


Figure 2. Aptamer activity in neuronal growth assays. A) 125 nM Nogo-66 blocks growth of embryonic cortical neurons. B) 125 nM Nogo-66 with 150 nM aptamer (Apt2-2; Nogo/lipid SELEX) stimulates growth.

Funding plan: These preliminary data will be included in a grant application to the NIH in response to the RFA: PA-15-071 from the NINDS. This opportunity falls under the Innovation Grants to Nurture Initial Translational Efforts (IGNITE) program specifically targeting neurological therapeutics. PA-15-071 requests “Pharmacodynamics and In vivo Efficacy Studies for Small Molecules and Biologics/Biotechnology Products.”

<http://grants.nih.gov/grants/guide/pa-files/PAR-15-071.html>