Controlled and Sustained Delivery of AG1478 for Optic Nerve Regeneration

Rebecca Robinson[1], Jun Lin[3], Cicely A. Williams[2], Jason M. Criscione[1], Stephen R. Viviano[4], James C. Tsai[3], and Erin B. Lavik[5].

[1]Yale University, Department of Biomedical Engineering; [2]Yale University, Interdepartmental Neuroscience Program; [3]Yale University School of Medicine, Department of Ophthalmology and Visual Science; [4]Yale University School of Medicine, Yale Vision Core Facility; [5]Case Western Reserve University, Department of Biomedical Engineering.

Statement of Purpose: Glaucoma is a group of neurodegenerative eye diseases typified by structural damage to the optic nerve that causes selective death of retinal ganglion cells (RGCs) leading to blindness. Early diagnosis and intervention can slow the progression of this disease; however current clinical therapeutic options fail to rescue or repair damaged RGCs. Recent work suggests that administration of the small-molecule epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor AG1478 promotes robust nerve regeneration and RGC survival. Adverse side effects of oral and systemic delivery of AG1478, make a single-dose, localized, minimally invasive administration of the treatment advantageous. In addition, pre-clinical studies have shown that to effectively inhibit EGFR sustained levels of AG1478 must be maintained. To address these needs we encapsulated AG1478 in poly(lactic-co-glycolic acid) (PLGA) microspheres. We hypothesized that local and sustained delivery of AG1478 would lead to increased regeneration in the injured optic nerve.

Methods: PLGA microspheres encapsulating AG1478, Coumarin-6—for tracking purposes—or no drug (blanks; control), were fabricated using a single emulsion technique with a co-solvent formulation of either 1:5 or 1:4 (dichloromethane:trifluoroethanol, DCM:TFE). Microsphere size was ascertained using a Multisizer™ 3 Coulter Counter® and confirmed visually via SEM. Loading and release of AG1478 microspheres was determined using UV-Vis at 330 nm. To confirm bioactivity of AG1478 after encapsulation, encapsulated and non-encapsulated AG1478 was added to FR3T3 cells in the presence of EGF. Cells were collected, lysed, and electrophoretically separated on reducing 6% SDS-polyacrylamide gels and then blotted for EGFR, phospho-EGFR, and α-Tubulin. Relative bioactivity was determined using the gray mean value for each phospho-EGFR band. After fabrication of microspheres and confirmation of bioactivity, AG1478 microspheres were administered in a rat optic nerve crush injury model to ascertain the effects on nerve regeneration. Briefly, the optic nerve was crushed for 10 s and 5 mins later a 5 μL volume of AG1478 microspheres suspended in 1X DPBS were injected into the sub-tenon space. At 1, 2, 4, and 7-week time points animals were sacrificed and the globe and nerve were dissected, cryo-sectioned and immunostained for markers of regeneration (e.g., GAP-43), gliosis (e.g., GFAP), and immune reaction (e.g., CD68).

Results: Microspheres were on average 2.56±1.90 μm in size. By increasing the ratio of DCM:TFE from 1:5 to 1:4 we were able to increase encapsulation from 65% (21 μg/mg polymer) to 76% (22 μg/mg polymer), respectively (Figure 1). Based on western blot analysis of activated EGFR, encapsulated AG1478 displayed the same

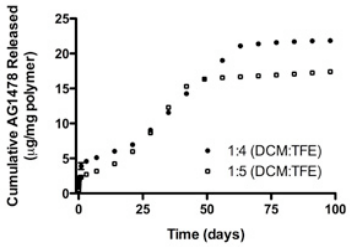

Figure 1. Cumulative release curves for AG1478 microspheres. Experiment performed in triplicate. Mean±SD

bioactivity *in vitro* as non-encapsulated AG1478 (89±2.7% and 89±4.5%, respectively; mean±SEM). Coumarin-6 microspheres were injected into the sub-tenon space to determine the location and persistence of microspheres. Coumarin-6 microspheres could be found proximal to the crush site as long as 7 weeks—the longest time point assayed—after injury. Administration of AG1478 and blank microspheres *in vivo* revealed significant differences in regeneration between the two groups. GAP-43 staining was higher in the optic nerve of animals that received AG1478 microspheres versus animals that received blank microspheres (Figure 2). In addition, regenerating fibers could be observed more than 1500 μm past the crush site. Analysis of GFAP and CD68 showed no differences between groups.

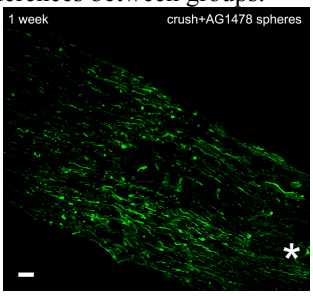

Figure 2. GAP-43 immunostained optic nerve. Asterisk indicates crush site. Scale = 100μm.

Conclusions: AG1478 can be encapsulated in PLGA microspheres and retain its bioactivity. We found that by increasing the amount of water-miscible solvent in the co-solvent ratio, the encapsulation can be significantly increased. Using a sub-tenon injection, microspheres persist for up to 7 weeks and deposit on the optic, near the crush site. Furthermore, administration of AG1478 microspheres greatly enhanced nerve regeneration compared to animals that received blank microspheres. Our findings suggest that local and sustained delivery of AG1478 using PLGA microspheres is a viable therapy for promoting neural regeneration for the treatment of glaucoma and CNS nerve injury more broadly.