Diabetic Complications Consortium

Application Title: Pre-clinical therapy delivery and imaging of nerve recovery in diabetic peripheral neuropathy of adipose and skin.

Principal Investigator: Kristy Townsend, Ph.D.

1. Project Accomplishments:

Over the past two years, we have undertaken the 2 Aims under this DIACOMP award (continued under an NCE due to COVID-related delays and an institutional relocation in fall 2020, from University of Maine to The Ohio State University). We have conducted the following studies with or without our microneedle device for gene therapy delivery of AAVs to overexpress neurotrophic factors, targeted to scWAT with the Rev2-AAV that is tropic for adipocytes: AAV-BDNF delivery to scWAT of HFD-induced mice (2 cohorts), AAV-BDNF or AAV-NGF to scWAT of HFD-induced mice (2 cohorts; the 2nd cohort is finishing now, both done with the microneedle device). We have optimized the pump-mediated delivery of the AAV through the microneedle array, and optimized the duration of the AAV delivery (4+ weeks is better), the empty virus vehicle control, and our panel of markers post-treatment (neurotrophic factors, synaptic markers, axon outgrowth markers, and neuroimmune markers). Tissue responses to the treatments have been variable, and thus we have concluded that: 1) a cocktail of neurotrophic factors may be desirable; and tropism for each may be important in order to appropriately 'wire up' nerves to their physiological cellular targets; 2) late in neuropathy severity, these particular treatments are less effective – thus, there may be a critical window for treatments and potentially also a 'point of no return' during disease progression; 3) microneedle delivery targets the viral vectors to the tissue of interest with minimal off-target expression; 4) neurotrophic factor Rec2-AAV delivery to scWAT of obese/neuropathic animals can improve metabolism under the right conditions (ie: increased thermogenic capacity, improved tissue innervation) but more tests will be needed to optimize the approach for the best results to improve animal health.

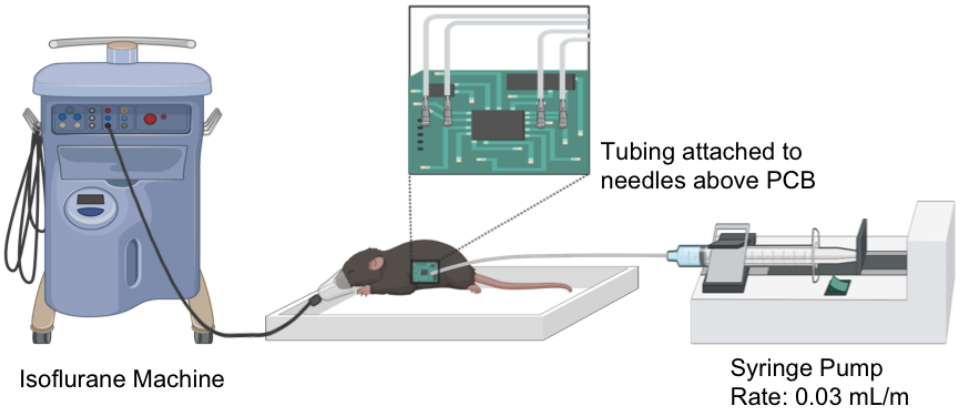
2. Specific Aims:

Aim 1: Utilize our novel microneedle device to deliver targeted AAV-BDNF therapies (tropic for adipocytes versus immune cells) to skin or scWAT in a diet-induced model of diabetic neuropathy, and determine therapeutic outcomes.

Results:

- Rec2-AAV-BDNF delivery to scWAT in diet-induced obese (high fat diet) neuropathic mice

Figure 1: **Schematic of microneedle device assisted delivery of gene therapy**



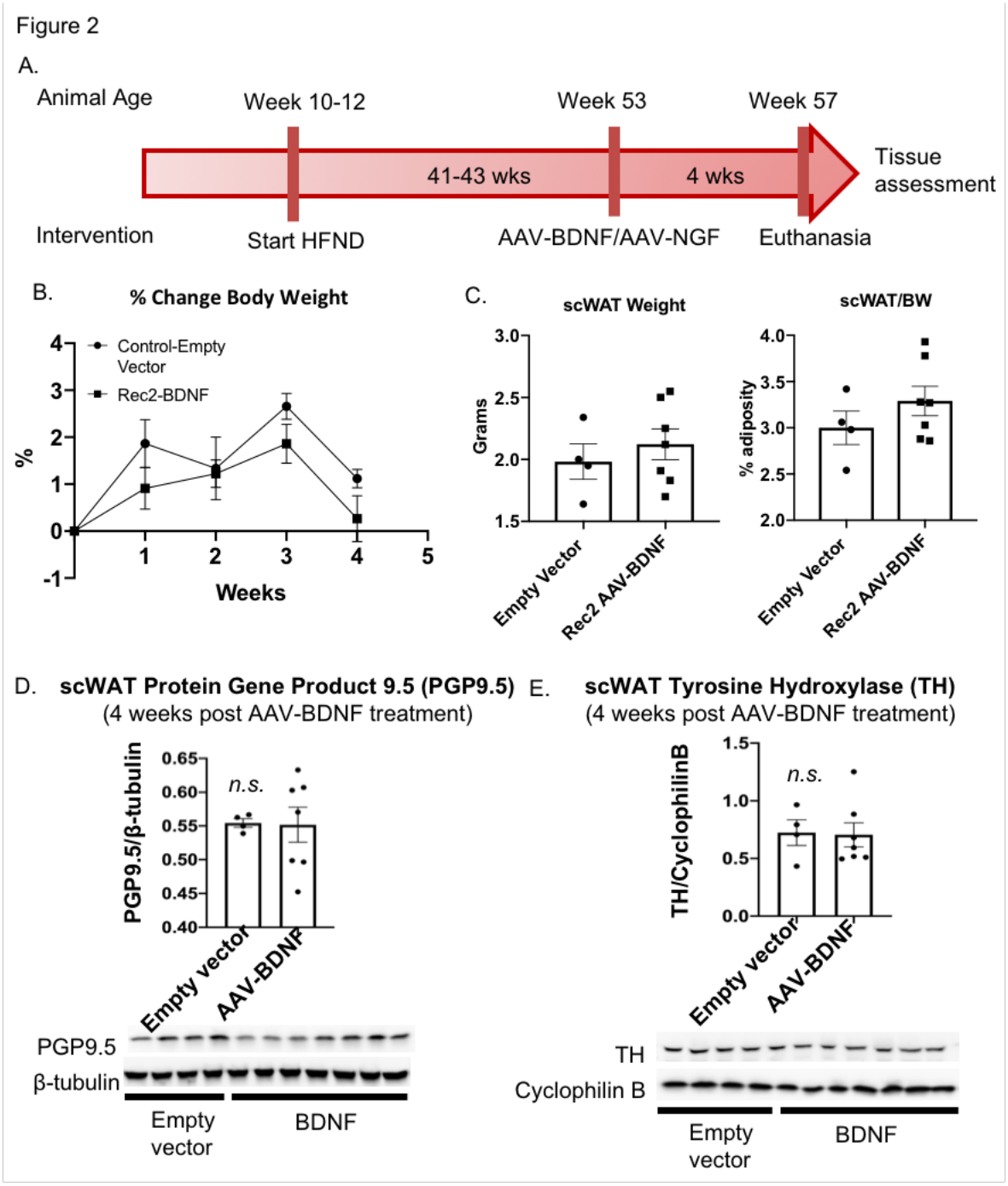


Figure 2: Adult male C57BL6 mice were placed on a 58% HFD for 41-43 weeks at which point Rec2-AAV-BDNF or an empty vector control was delivered to inguinal scWAT via microneedle device at a dose of $1x10^{10}$ vg. Following gene delivery, animals were maintained on HFD for an additional 7 weeks. (A) Timeline of HFD feeding and AAV mediated BDNF or empty vector treatment. (B-C) By 4-weeks post treatment both groups had similar body weight. (D-E)

There was no difference in protein expression of PGP9.5 (D) and tyrosine hydroxylase (TH) (E) in inguinal scWAT at 4-weeks post between Rec2-AAV-BDNF treatment and control groups.

- Rec2-AAV-BDNF or Rec2-AAV-NGF delivery to scWAT in diet-induced obese (high fat diet) neuropathic mice

Figure 3:

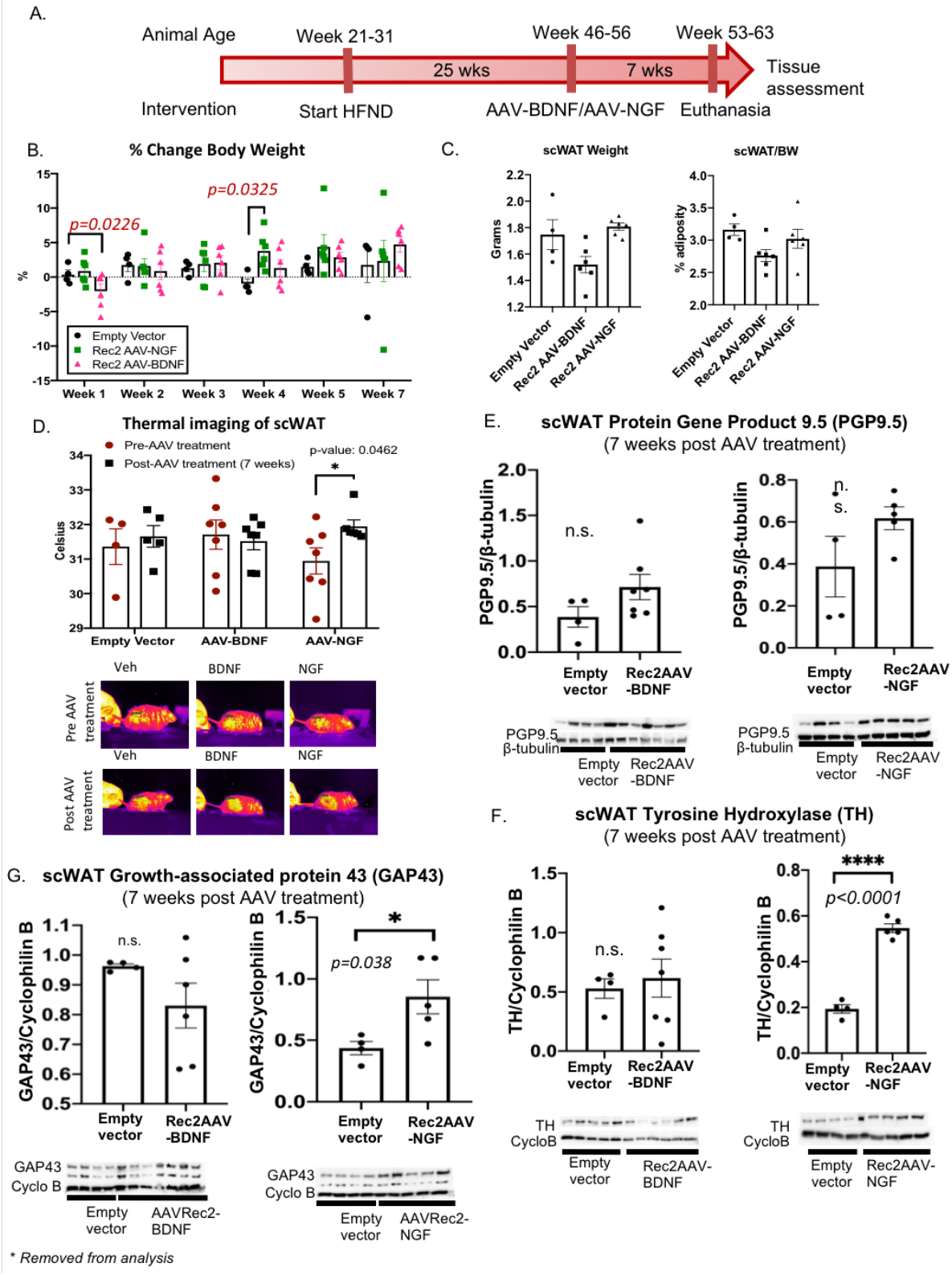


Figure 3: Adult male C57BL6 mice were placed on a 58% HFD for 24 weeks at which point Rec2-AAV-BDNF, Rec2-AAV-NGF or an empty vector control was delivered to inguinal scWAT via microneedle device at a dose of $1x10^{10}$ vg. Following gene delivery, animals were maintained on HFD for an additional 7 weeks. (A) Timeline of HFND feeding and AAV mediated BDNF or NGF treatment. (B-C) By 7-weeks post treatment all groups had similar body weight. (D) Mice that had received Rec2-AAV-NGF treatment showed increased skin surface temperate above the inguinal scWAT 7-weeks post treatment as evidenced by thermal imaging, suggesting increased thermogenic activity/browning. (E) Compared to empty vector controls neither Rec2-AAV-BDNF nor Rec2-AAV-NGF treatment increased protein expression of PGP9.5 in inguinal scWAT at 7-weeks post treatment. (F) Protein expression of TH was greatly increased in scWAT of only Rec2-AAV-NGF treated mice compared to empty vector controls , which corresponds to the thermal imaging data in (C). (G) Protein expression of GAP43, an axonal growth cone protein and marker of neurite outgrowth, was also measured in scWAT 7-weeks post AAV treatment. Again, only AAVRec2-NGF treatment resulted in an increase in GAP43 expression compared to empty vector controls.

Aim 2: Interrogate skin and adipose neurite density after optimal AAV-BDNF therapy using novel whole-tissue imaging and molecular analyses; comparing males and females with diet- or genetic-induced diabetic neuropathy.

Results: We are currently processing skin and adipose samples from the cohorts in Aim 1; data from both Aims is currently being placed into the In Prep manuscript listed below under Publications.

3. Publications:

1. Blaszkiewicz, M, K Mensah-Ahrin, N Storey, T Tao, L Cao, KL Townsend*. Microneedle device-mediated delivery of gene therapy targeting subcutaneous adipose tissue adipocytes for the treatment of diabetic peripheral neuropathy. (In Prep, planned submission to Diabetes in Dec 2022). *Corresponding author