1. PURPOSE
Non-clinical and clinical studies have demonstrated the preliminary safety and efficacy of a novel implant device (NT-503-3) (Figure 1) encapsulating a retinal pigment epithelial cell line (NTC-203-910) engineered to continuously produce a homodimer, Fc chimeric, VEGF inhibitor (910-VEGFR). Analytical techniques, including proteomic mass spectroscopy (MS), were used to characterize the 910-VEGFR protein and identify the most abundant proteins naturally secreted from the encapsulated NTC-203-910 cell line.

2. METHODS AND MATERIALS
5 devices from 3 consistency lots of NT503-3 product, one week post encapsulation were incubated in culture media for 24 hours. Device conditioned media (CM) was analyzed by VEGF direct binding ELISA, SDS PAGE gels and western blots and MS.

For MS analysis CM samples were buffer exchange with a 0.5 ml, 3 kDa Amicon filter (Millipore). Nano-LS/MS/MS using a trapping configuration with a 4 hour reverse phase gradient identified the most abundantly secreted proteins by the encapsulated cell line. Relative abundance of each protein (NSAF) was determined by normalizing raw MS spectral counts (SpC) for molecular weight and calculating relative percentage of total spectral counts (NSAF). Standard statistical methods, mean, standard deviation (SD), percent coefficient of variation (%CV), and coefficient of determination (R²) compared the NSAF profile of secreted proteins from the 15 NT-503-3 devices.

3. RESULTS
3.1. Intralot and Interlot Variation MS Total SpC
Vascular endothelial growth factor (VEGF) direct binding ELISA determination of 910-VEGFR output was within the specification range of manufacturing product stability. The mean 910-VEGFR released per lot (n=5) was 10,831±610, 9,355±1,445, and 11,101±1508 ng/device/day.

3.2. MS Total SpC counts were compared within and between lots. The intralot and interlot variation is <14% (Figure 3). 352 proteins were reliably identified technically with the 4 hour gradient mass spec method employed (SpC>5). These proteins accounted for over 90% of the proteins. NSAF values indicating relative abundance for the 352 proteins were calculated showing 910-VEGFR is the most abundant protein secreted. Pair-wise linear regression analysis of the protein profile (NSAF) resulted in R² values >93% indicating stable protein expression among devices and lots of NT503-3 product (Figure 4).

3.3. MS Total SpC values were compared within and between lots. The intralot and interlot variation is <14% (Figure 3). 352 proteins were reliably identified technically with the 4 hour gradient mass spec method employed (SpC>5). These proteins accounted for over 90% of the proteins. NSAF values indicating relative abundance for the 352 proteins were calculated showing 910-VEGFR is the most abundant protein secreted. Pair-wise linear regression analysis of the protein profile (NSAF) resulted in R² values >93% indicating stable protein expression among devices and lots of NT503-3 product (Figure 4).

3.4. Comparison MS protein profile. For SpC comparison, all the detected proteins were compared. For NSAF comparison, the 352 most reliably detected technically proteins were compared. Six clinical lots (n=3 devices/lot) were examined by the same MS technique. The protein profiles (NSAF of 352 technically reliably identified proteins) were compared to the consistency lots using the same pair-wise linear regression analysis. The R² for the secreted proteins was greater than 0.9 suggesting a consistency in the quality and quantity of the most abundantly secreted proteins from NT503-3 devices (Figure 5).

4. CONCLUSIONS
A single, intracocular ECT implant delivering 910-VEGFR was designed to provide comparable anti-VEGF therapy to standard-of-care treatments while eliminating the burden of frequent injections in patients with neovascular AMD. MS proteome profiling identified, in addition to 910-VEGFR, the most abundant proteins stably secreted by NT-503-3 devices as lower-level, naturally occurring proteins normal to human retinal pigment epithelial cell line metabolic, development and organizational function (Figure 6).

The authors would like to acknowledge MS Bioworks, based in Ann Arbor, Michigan for excellent technical services.

L. Orecchio 1, M. Rivera 1, E. Brissette 1, C. McGovern 1, A. Nystuen 1, K. Kauper 1
Neurotech Pharmaceuticals Inc., Cumberland, RI

Lisa Orecchio
lorechio@neurotechusa.com