

# Molecular Characterization of VEGF Secretion by MSC-NTF Cells (NurOwn®): Therapeutic Implications in ALS

Haggai Kaspi, Revital Aricha, Natalie Abramov, Yael Gothelf, Chaim Lebovits, Joseph Petroziello, Yossef S. Levi, Ralph Kern

BrainStorm Cell Therapeutics, Petach Tikvah, Israel and New York City, NY, USA



## Background

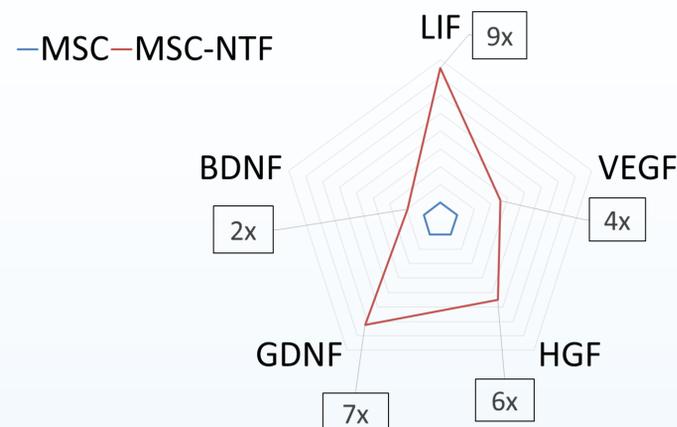
MSC-NTF cells (NurOwn®) are autologous bone marrow derived mesenchymal stem cells (MSC) induced in culture to secrete high levels of neurotrophic factors (NTFs) that support neuronal growth and survival. Thus, MSC-NTF cells combine MSC's immunomodulatory therapeutic benefits with enhanced neurotrophic factor secretion. A phase 3 randomized clinical trial is in progress at 6 sites in the US to evaluate the efficacy and safety of repeated intrathecal administration of MSC-NTF cells in ALS patients. As previously reported, VEGF has an important immunomodulatory and neuroprotective role in ALS through biological effects on microglia and glutamatergic disease mechanisms.

## Methods

To explore the molecular differences between naïve MSC and MSC-NTF cells, we performed mass-spectrometry proteomics, Affymetrix gene array and microRNA array. To map changes in protein secretion, ELISA assays were employed. Subsequently, several bioinformatic tools were applied to investigate the interactions between protein secretion, protein/gene expression and microRNAs.

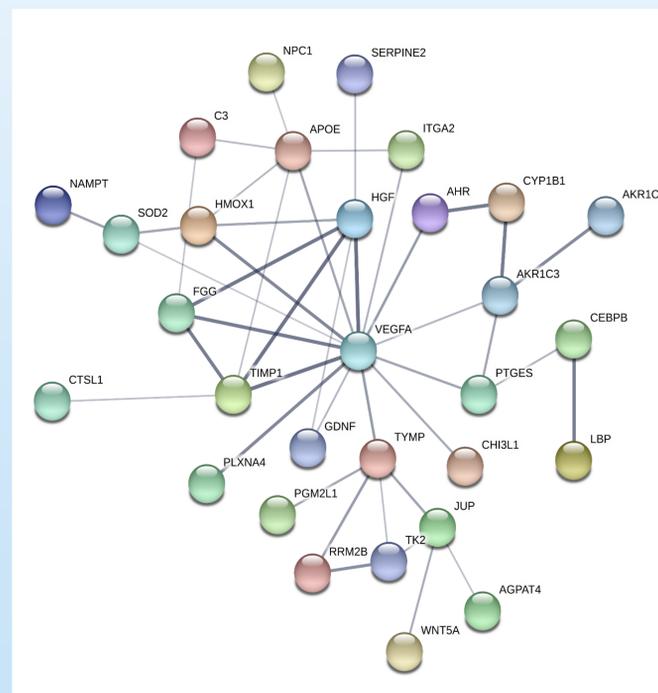
## Results

### Increased secretion of multiple NTFs by MSC-NTF cells *in-vitro*



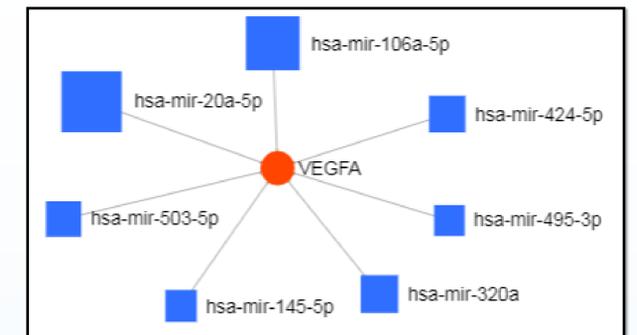
A radar plot depicts the fold change in secretion of selected neurotrophic factors by MSC-NTF in comparison to MSC. Protein abundance was measured by ELISA. n=28 patients for all factors except LIF (n=18).

### VEGF is intertwined with proteins which are upregulated in MSC-NTF



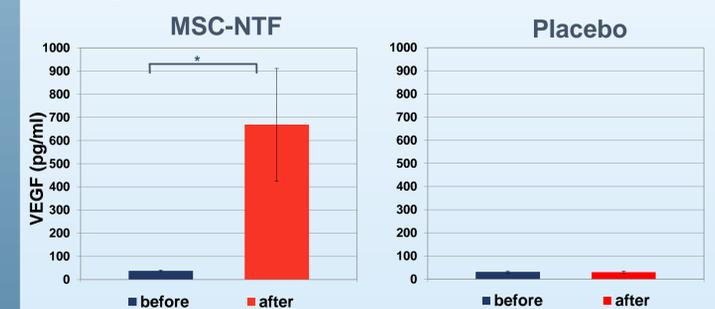
STRING analysis reveals interactions of VEGF with proteins which were found to be significantly upregulated in MSC-NTF vs. MSC by mass-spectrometry. Thicker line depicts stronger predicted interaction. Aryl hydrocarbon receptor (AHR) which was previously shown to upregulate VEGF expression was found to be significantly upregulated in MSC-NTF vs. MSC.

### Changes in miRNA expression support increased VEGF secretion by MSC-NTF



Twenty-two microRNAs were found to be significantly down regulated in MSC-NTFs vs. MSCs (pFDR<0.05, FC>1.5). Of these, seven microRNAs (depicted in the figure above) are predicted by the miRNet algorithm to target VEGF. Thus, down-regulation of these miRNAs should contribute to increased expression and secretion of VEGF.

### Upregulation of VEGF in the CSF of patients treated with MSC-NTF



VEGF ELISA of CSF samples taken from patients before or two weeks after MSC-NTF/placebo intrathecal transfusion. (n=26 for MSC-NTF and n=9 for placebo, \*p<0.05).

## Conclusions

There are significant differences in protein and microRNA expression between MSC and MSC-NTF cells that may contribute to NurOwn®'s mechanism of action. Changes in microRNA expression are likely to contribute to increased VEGF secretion in MSC-NTF cells. The significantly increased secretion of VEGF and other NTFs by MSC-NTF cells may contribute to key ALS neuroprotective and immunomodulatory therapeutic mechanisms.