BACKGROUND. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects upper and lower motor neurons (MN) leading to muscle atrophy and paralysis. Within 3-5 years from diagnosis, patients succumb due to respiratory failure (1). ALS is a non-cell autonomous disease, in which microglia and astrocytes play a central role in the clinical progression secreting neurotoxic and pro-inflammatory factors (2). In a previous work we investigated the effect of intravenous administration of mesenchymal stem cells (MSCs) in SOD1G93A mice (3). A single injection of MSCs determined survival improvement, improvement of motor skills and reduction of gliosis and inflammation in spinal cord. These beneficial effects were determined by panetraxic mechanisms, rather than MSC differentiation. We have speculated that MSCs exert their action in part through the transfer to target cells of miRNA shuttled by their released exosomes.

AIM. The objective of this study was to verify whether exosomes derived from IFN-1-primed MSCs and their shuttled miRNAs modulate the phenotype of cultured astrocytes prepared from the spinal cord of late symptomatic SOD1G93A mice and reduce the inflammatory environment surrounding MN. We analyzed astrocyte activation state, cytokine expression and release, the impact of astrocytes on MN viability in astrocyte/MN co-cultures.

RESULTS

1. MSCs and Exosome Characterization
Nine miRNAs are up-regulated in MSCs activated by IFN-1 (not shown). These miRNAs were also expressed in exosomes.

2. Astrocyte Activation
The expression of the astrocyte-mediated inflammation marker NLRP3 was significantly increased in spinal cord astrocytes from adult SOD1G93A spinal mice. NLRP3 overexpression was reduced after exosome treatment.

3. Pro-Inflammatory Markers
The expression of TNF-α (A), IL-1β and IL-6 was quantified by immunofluorescence in spinal cord astrocytes from adult SOD1G93A mice. The 466G, 467F, 466F5p, 512S or 3082S5p synthetic miRNA transfection reduced the over-expression of TNF-α and IL-1β.

4. NLRP3
The expression of the anti-inflammatory cytokine IL-10 was significantly decreased in spinal cord astrocytes from adult SOD1G93A mice. IL-10 down-regulation was normalized after exosome treatment.

5. Motor Neuron Survival
MN viability was analyzed in presence of spinal cord astrocytes from adult SOD1G93A mice, that were treated or not with exosomes for 24 hours. Preliminary data showed an improvement of survival of MNs in the presence of astrocytes treated with exosomes, when compared to co-culture with untreated astrocytes.

HIGHLIGHTS
- Exosome treatment reduced the SOD1G93A astrocyte activated phenotype.
- The expression of pro-inflammatory cytokines, increased in SOD1G93A astrocytes, was reduced in exosome-treated SOD1G93A astrocytes.
- Synthetic miRNAs mimicked the beneficial effects of exosomes in SOD1G93A astrocytes.
- Treating astrocytes with exosomes had a positive impact on motor neuron viability.