**Potential and immuno-modulant proprieties of mesenchymal stem cells from amniotic fluid** Katia Mareschi<sup>1, 2</sup>, Deborah Rustichelli<sup>1</sup>, Michela Muraro<sup>1</sup>, Sara Castiglia<sup>1</sup>, Edoardo Errichiello<sup>1</sup>, Elena Signorino<sup>1</sup>, Franca Fagioli<sup>1</sup>.

<sup>1</sup> Stem Cell Transplantation and Cellular Therapy Unit; Pediatric Onco-Hematology Department,

Regina Margherita Childrenøs Hospital, Turin, Italy

<sup>2</sup> Department of Pediatrics ó University of Turin

<sup>3</sup> Department of Neuroscience, NIS Centre, University of Turin, Italy

<sup>4</sup> Department of Gynecology and Obstetrics, University of Turin, Italy

Amniotic fluid (AF) contains stem cells which, due to their ontogenetic origin, have a high proliferative and differentiative potential and might be an attractive source of multipotent stem cells for therapeutic transplantation. We studied the phenotypic characteristics, differentiation potential and immunomodulation proprieties in vitro of mesenchymal stem cells (MSCs) isolated from human amniotic fluid (AF), during routine prenatal amniocentesis, and evaluated their compared to bone marrow (BM) MSCs.

We isolated multi-potent stem cells from AF that showed a high proliferative potential, were positive for CD90, CD105, CD29, CD44, CD73, CD166; showed Oct-4 and Nanog molecular and protein expression and differentiated into osteoblasts, adypocytes and chondrocytes. In Neural Progenitor Mantainance Medium (NPMM) AF-MSCs expressed neural markers and increased Na+ channel density. Inactivation of the TTX-sensitive channels accelerated and became more similar to native neuronal voltage-gated Na+ channels.

We investigated the beneficial immunomodulatory effects of MSCs in co-cultures with different immunogenic populations such as Dendritic Cells (DCs) and regulatory CD4+ CD25+ T cells.

MSCs strongly inhibit the differentiation of monocytes to DCs and there was a significant reduction in the expression of CD83 mature DCs treated with MSCs, suggesting their skew to immature status. A decreased expression of presentation molecules (HLA-DR) and co-stimulatory molecules (CD80 and CD86) were also observed.

CD4+CD25+ regulatory T cells significantly increase in presence of MSCs. In the control group there is 5.5% of CD4+CD25+ T cells, 23.4% in co-culture with AF-derived MSCs, and 16% in co-culture with BM-derived MSCs.

These data suggest that AF-MSCs have a stronger immunosuppressive effect compared to BM-MSCs. This interesting aspect can be easily due to the more premature stemness stage of the AF-MSCs rather than BM-MSCs. We hypothesise that MSCs have proliferative, differentiative and immunological characteristics more similar to AF-MSCs than to BM-MSCs.

AF is an important multipotent stem cell source with a high proliferative potential able to originate potential precursors of functional neurons and showed a stronger immunosuppressive effect compared to BM-derived MSCs. This effect was mediated by inducing the generation of CD4+CD25+ regulatory cells and by suppressing monocyte differentiation into DCs, thus indicating the important role of AF-MSCs on immunoregulation