



**28<sup>E</sup> CONGRES DU CHO**  
**12-15 OCTOBRE 2022**  
**PRESQU'ILE DE GIEN**



**POSTER 10: In vitro expansion and gene modulation of murine HSCs for improvement of immunotherapy against solid malignancies**

Laura Falceto Font 1, Dan Jin 1, Connor Francis 1, Bayli Divita Dean 1, Brianna McDonald 1, Catherine Flores 1.

1: University of Florida

Primary murine hematopoietic stem ceUs (mHSCs) have been historically challenging to grow in vitro for long-term culture due to the short periods of time they can remain at the self-renewal and undifferentiated state. Furthermore, this has provided an extra challenge for the genetic modification of mHSCs that adds to the inherent difficulty of modifying slowly cycling and quiescent primitive HSCs. In the last few years, there has been an effort to optimize the culture conditions of mHSCs for successful gene editing. Here, we test and validate different in vitro conditions for expansion of mHSCs including growth media and volume, cell number, duration of the culture, and culture plate size conditions. We then employ various methods to genetically modulate mHSCs including shRNA, CRISPR, and AAV. This study describes the efficacy of each method as well as the observed limitations of each technique on mHSCs. Here, our purpose was to drive mHSCs into immune activating dendritic ceU lineage ceUs, or alternatively preventing suppressive myeloid-derived suppressor cells to arise from the mHSCs. Ultimately, these genetically modified cells will be used as an adjuvant therapy to improve the efficacy of various immunotherapeutic strategies against solid malignancies.

