Based on the research findings highlighted by La Greca et al., the proteomic composition of extracellular vesicles (EVs) derived from induced pluripotent stem cells (iPSCs), iPSC-derived mesenchymal stem cells (iPSC-MSCs), and conventional mesenchymal stem cells (MSCs) varies significantly. This suggests a nuanced evolution of protein content as iPSCs transition into iPSC-MSCs, with the resulting EVs exhibiting a distinct proteomic signature that is more specific and likely reflects their specific functions within the stem cell microenvironment. This includes roles in supporting stem cell maintenance, facilitating differentiation, and mediating intercellular communication within tissues.

Given these insights, our approach will involve harnessing iPSC-MSCs to generate EVs for therapeutic purposes. To meet the demands for high yield and potency necessary for clinical applications, we will employ innovative culture strategies, particularly bioreactors, which offer continuous culture capabilities and enable real-time monitoring of crucial parameters such as oxygen levels and pH. Recent work by Cao et al. has demonstrated that EVs derived from 2D cultures and hollow fiber bioreactor (HFB)-cultured MSCs exhibit comparable surface marker profiles, size, and morphology, with the latter yielding up to a 19.4-fold increase in production.

For the culture of EVs, our methodology will involve utilizing a bioreactor system with a 48-hour harvest interval supplemented with human platelet lysate (HPL) as a culture medium. HPL not only supports xeno-free MSC culture, aligning with clinical trial requirements, but also enhances translational potential. It's worth noting that while HPL contains exogenous serum derived EVs along with other nanoparticles such as growth factors and protein aggregates, it still represents a superior serum alternative within this context.

After the EVs have been released into the culture medium, for EV isolation, we will use a microfluidic system which can isolate exosomes with high purity, minimizing contamination form other extracellular vesicles or protein aggregates. The process is more efficient and requires less time than ultracentrifugation techniques (gold standard), it can be scaled up and the same system can be used for exosome modifications.

Using Western Blot or Elisa, we will then proceed to the characterization of the MSCs assessing the assessment of protein markers, including CD9, CD63, CD81, CD59, as well as cytosolic proteins such as ALIX, TSG101, and Hsp70/90.

To enhance the specificity of iPSC-MSC-derived exosomes for targeting tumor-associated macrophages (TAMs) and cancer cells, we must delicately balance the need for efficient TAM targeting with the imperative to evade uptake by macrophages and leukocytes in the Mononuclear Phagocyte System (MPS) organs. This optimization is critical to ensure that the engineered exosomes maintain sufficient circulation time to effectively reach and target tumors.

Considering the limitations associated with PEGylation, we are exploring alternative strategies such as "Self" peptide conjugation. This approach aims to modify the exosome surface with self-peptides that mimic endogenous proteins, thereby potentially reducing recognition by MPS cells while preserving exosome integrity and targeting specificity:

* **Combine CD47 expression:** with other targeting ligands specific to TAMs. By incorporating TAM-specific ligands on the exosome surface, such as antibodies against TAM markers (e.g., CD68, CD206, CD163), the exosomes can selectively target TAMs despite CD47-mediated immune evasion. We will also conjugate the exosome surface with CSF-1R inhibitors which have shown to lead to enrichment in the M1 population, in addition to altering TAM recruitment and distribution However, the largest macrophage polarization effects have been observed for agonists of the toll-like receptors 7 and 8 (TLR7/8), and we may want also to investigate these therapeutics. These inhibitors are small molecules which can be modified to introduce a thiol-reactive group, such as a maleimide function group. The isolated exosomes can then be functionalized with thiol groups on their surface, providing sites for conjugation with the maleimide-linker, conjugating the drug to the exosomes.
* **Optimization of CD47 Expression Levels**: Modulate the expression levels of CD47 on exosomes to balance immune evasion with TAM targeting. Fine-tuning CD47 expression can potentially optimize exosome biodistribution and maximize TAM targeting.

Lipids such as phospholipids or cholesterol derivatives can be modified to include pH-sensitive functional groups, such as imidazole or carboxylic acid moieties. These lipids can be incorporated into the lipid bilayer of the exosomes. At physiological pH, the lipid bilayer remains intact, providing stability to the exosomes. However, under acidic conditions in the tumor microenvironment, the pH-sensitive groups undergo protonation or deprotonation, causing changes in lipid bilayer structure and facilitating cargo release.