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of CD105 +, CD34- , CD73 +, CD90 +, CD45- .

| Characteristics of patients | | |
|-----------------------------|---------------|------------|
| Patients with EM | | n: 8 |
| Age | mean (DE) | 46,37(5,7) |
| | Range (years) | 40 - 54 |
| | | |
| Gender | female | 7 (87,5%) |
| | Male | 1 (12,5%) |
| EDSS | Score 1 a 3 | 62,5% |
| | Score >3 a 5 | 37,5% |
| | Range | 0 - 5 |
| Medicamento n (%) | Interferón-β | 5 (62,5%) |
| | Not treatment | 2 (25%) |
| | Fingolimob | 1 (12,5%) |

Table 2. Stimulation with PHA. Median LT proliferation and cytokines in the supernatant of co-cultivation.

| MEASUREMENT | PARAMETER | PHA | PHA MSC1:1 | PHA MSC1:10 | p. |
|----------------------|---------------|-----------------|---------------|-----------------|-------|
| PROLIFERATION | Median % | 80,8 | 77,35 | 66,55 | 0,368 |
| | Interquartile | | | | |
| | Range | 61,92-91,17 | 58,57-86,45 | 39,5-91,62 | |
| INF | Median pg/ml | 595,72 | 357,95 | 3426,66 | 0,034 |
| | Interquartile | | | | |
| | Range | 548,11-6984,63 | 133,01-909,53 | 875,98-8.376,74 | |
| TNF | Median pg/ml | 2309,24 | 156,23 | 3924,78 | 0,011 |
| | Interquartile | | | | |
| | Range | 171,58-5.996,64 | 20,87-166,07 | 832,24-5.653,22 | |
| IL10 | Median pg/ml | 74,33 | 36,48 | 66,34 | 0,494 |
| | Interquartile | | | | |
| | Range | 0-154,1 | 21,08-81,46 | 46,83-120,88 | |
| IL6 | Median pg/ml | 2449,39 | 23805,91 | 23.805,91 | 0,001 |
| | Interquartile | | | | |
| | Range | 431,17-6.047,5 | 23.805,91 | 3.805,91 | |
| IL4 | Median pg/ml | 46,69 | 0 | 0 | 0,033 |
| | Interquartile | | | | |
| | Range | 0-49,61 | 0 | 0-32,32 | |
| IL2 | Median pg/ml | 296,08 | 0 | 22,88 | 0,043 |
| | Interquartile | | | | |
| | Range | 0-3.602,59 | 0 | 0-901,62 | |

Table 3. Stimulation with CD3 / CD28. Median LT proliferation and cytokines in the supernatant of co-cultivation.

| MEASUREMENT | PARAMETER | CD3/28 | CD3/28 MSC1:1 | CD3/28 MSC1:10 | p. |
|----------------------|---------------|-------------------|-------------------|-------------------|-------|
| PROLIFERACIÓN | Median % | 26 | 64,95 | 32,75 | 0,026 |
| | Interquartile | | | | |
| | Range | 13,32-34,7 | 31,25-90,22 | 26,45-49,42 | |
| INF | Median pg/ml | 1.821,86 | 9.906,57 | 1974,3 | 0,003 |
| | Interquartile | | | | |
| | Range | 918,59-2.657,39 | 9.589,67-10.899,6 | 1.390,39-4.963,43 | |
| TNF | Median pg/ml | 1462,24 | 6147,35 | 3357,54 | 0,001 |
| | Interquartile | | | | |
| | Range | 1.130,35-1.982,75 | 5.662,35-9.431,21 | 2.450,06-4.955,09 | |
| IL10 | Median pg/ml | 24,6 | 75,96 | 30,82 | 0,111 |
| | Interquartile | | | | |
| | Range | 0-36,63 | 30,73-110,27 | 0-69,29 | |
| IL6 | Median pg/ml | 691,29 | 23.805,91 | 23.805,91 | 0,001 |
| | Interquartile | | | | |
| | Range | 214,06-1.094,48 | 23.805,91 | 23.805,91 | |
| IL4 | Median pg/ml | 9,22 | 0 | 0 | 0,112 |
| | Interquartile | | | | |
| | Range | 0-29,93 | 0-5,51 | 0 | |
| IL2 | Median pg/ml | 984,6 | 528,39 | 1106,7 | 0,823 |
| | Interquartile | | | | |
| | Range | 130,2-11.864,96 | 107,94-6.433,62 | 261,77-4.336,62 | |

DISCUSSION

The induction of proliferation was observed in the presence of two concentrations of MSC in different stimuli (CD3/CD28 and Myelin), contrasting with that data reported by Zaforskaya et al. (2013) who demonstrated that MSCs can inhibit response of T lymphocytes in MS patients, triggered by the mechanism of IFN-γ and indoleamine-2,3-dioxygenase (IDO). Similarly, Bai et al. (2009) shows that this decreases proliferation and promotes Th2 profile under similar conditions.

In this study was the increased concentration of IL-6 in the supernatant of the co-cultures in the presence of MSC with PHA, CD3/CD28 and myelin. For example, using myelin (0,93μg/ml) as a stimulant, the baseline concentration of IL-6 increased 855 times in the presence of MSC 1: 1 compared with cultures in the absence of MSC. A similar behaviour is given to the other two concentrations of myelin (3,75μg / ml and 15μg / ml), which increased 211 y 168 times respectively. The increase of the IL-6 can be explained by different non-exclusive theories: 1. Using total mononuclear cells, which include antigen-presenting cells that may stimulate response in "naive" CD4 T cells. It is further known that high concentrations of IL-6 participate in Th17 differentiation (Bai et al 2009). 2. The expression of IL-6 constitutively formed in the MSC with and without stimulus. (Melief et al. 2013).

It is important for future studies that healthy individuals are included as control groups, which will allow us to determine whether the results are influenced or not by the disease in patients with RRMS. Besides the measurement of IL-17, since this is a major cytokines associated with disease pathogenesis. Finally it is essential to decrease the heterogeneous population of MSC, so it is suggested using populations MSC enriched with CD271 or CD146 population, which have shown an immunomodulatory profile or been associated with a perivascular phenotype respectively (Zyrfete et al. 2011, Tormin et al. 2011).

Conclusion:

A modified pattern of secretion of inflammatory cytokines in the presence of MSC, contrasting with the results showing that they cause inhibition dose-dependent proliferation, was observed.

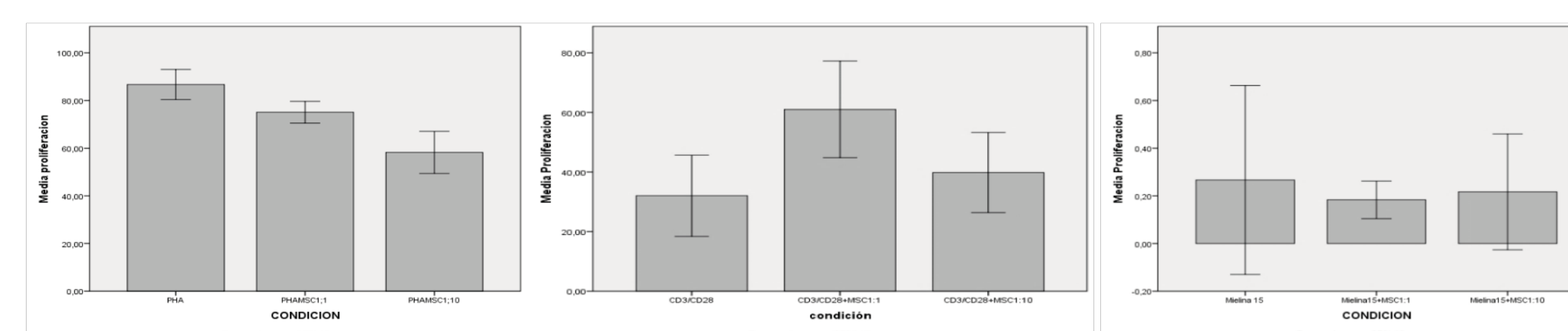
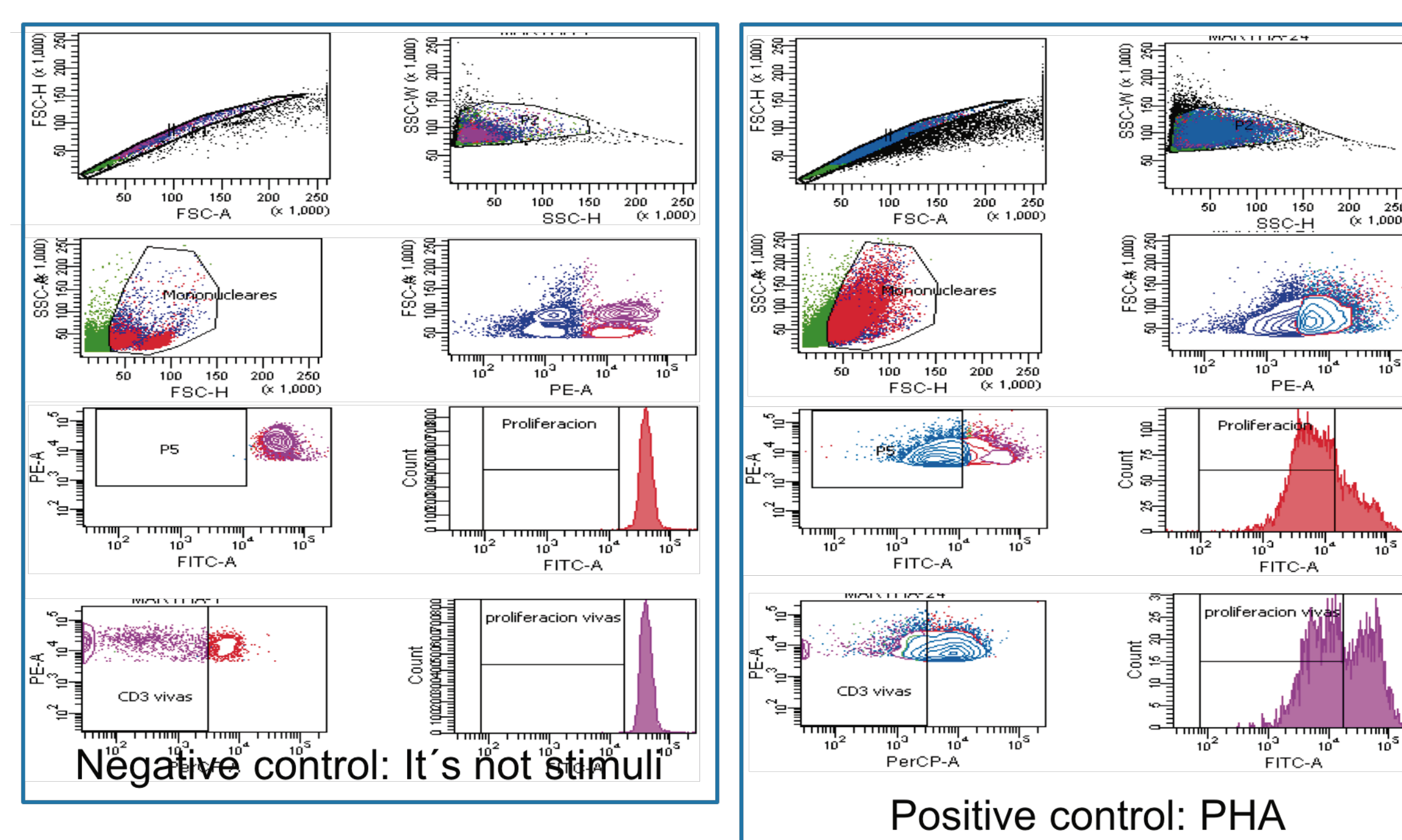
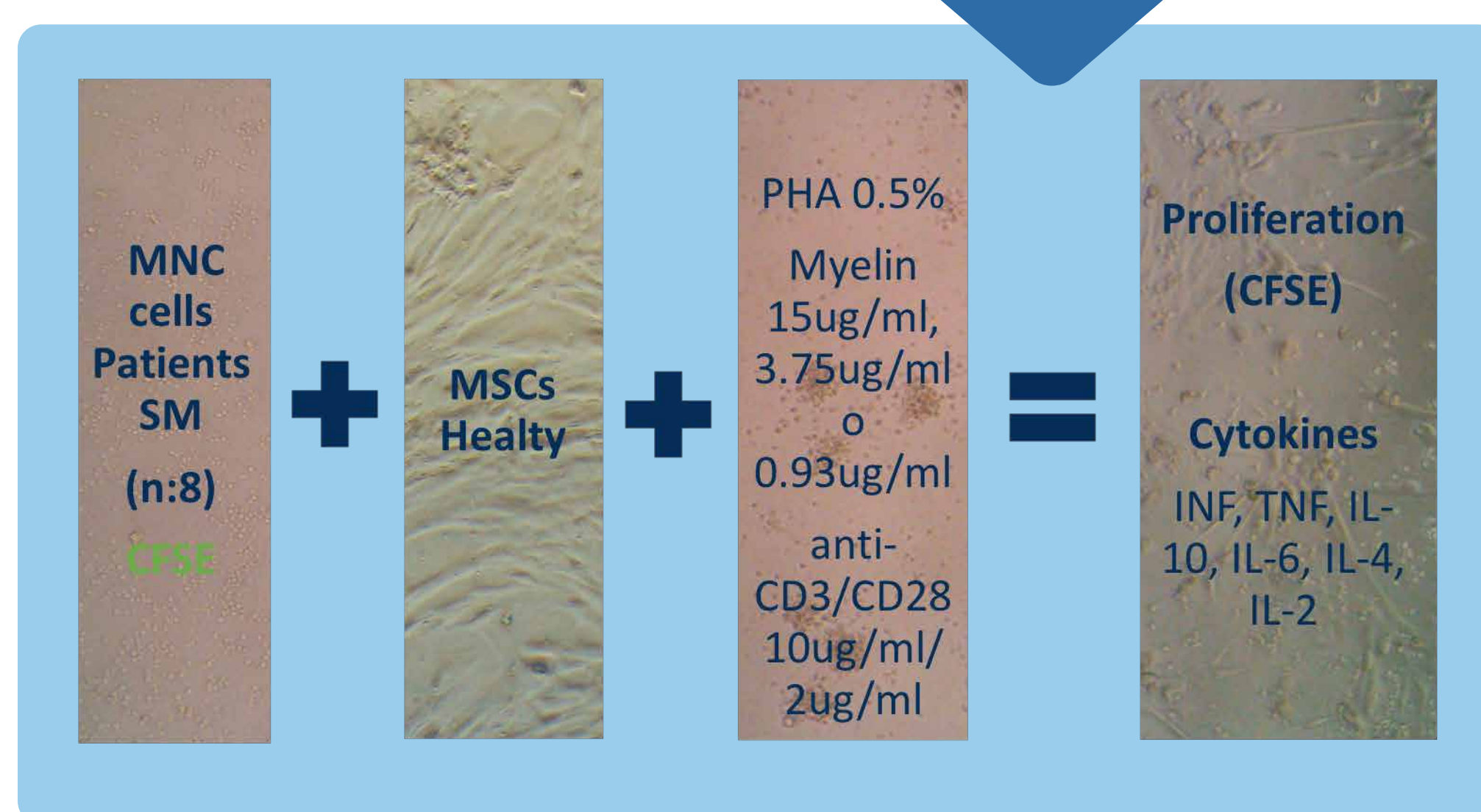
Therapy with Mesenchymal Stem Cells (MSC), evaluated in several clinical trials with different autoimmune diseases, has been found to have conflicting results with regard to its effectiveness. The objective was determine whether there is a change in the growth and pattern of cytokine secretion in response to the lymphocytes T (LT) from patients with Relapsing-Remitting Multiple Sclerosis (RRMS) when subjected to different stimuli in co-culture with allogeneic MSC

Methodology:

The MSC in two proportions 1:1 and 1:10 were co-cultured with mononuclear cells (MNC) from RRMS patients to determine their immunomodulatory effect. Measurement of proliferation was performed by carboxyfluorescein diacetate succinimidyl ester (CFSE) and cytokine solubles TH1/TH2 through CBA (Cytometric Bead Array BD*).

Obtaining MSCs

The bone marrow samples from healthy individuals were obtained by puncture of iliac crest and . Test bone differentiation and Adipogenic differentiation. MSCs cells were frozen. Descongelamiento de MSCs



A) Proliferation. PHA: Proliferation of 86.68% of which decreased in the presence of MSCs1: 1 at an average of 75.08% and an average proliferation in the presence of MSCs1: 10 of 58.25%. B) CD3/8: Proliferation of 32.03%, which increased in the presence of MSCs1: 1 at an average of 61.01% and an average proliferation in the presence of MSCs1: 10 of 39.82%. C) Myelin 15μg / ml: Proliferation of 0.26%, which increased in the presence of MSCs1: 1 at a mean of 0.18% and an average proliferation in the presence of MSCs1: 10 of 0.21% D) Myelin 3.75μg / ml: An increase in proliferation of 0.09% (± 0.13) was found which increased in the presence of MSCs1: 1 to an average of 0.93% (p. 0.002) and to a 0.11% (± 1.2) in the presence of 1:10 MSC. E) Myelin 0.93μg / ml: An increase in proliferation of 0.02% (± 0.13) was found which increased in the presence of MSCs1: 1 to an average of 0.73% (p. 0.002) and to a 0.10% (± 1.2) in the presence of 1:10 MSC.

Table 1. Stimulus Myelin. Median LT proliferation and cytokines in the supernatant of co-cultivation.

| MEASUREMENT | PARAMETER | MYELIN 15UG/ML | MSC1:1 | MSC1:10 | p. |
|----------------------|---------------|----------------|--------------------|--------------------|--------|
| PROLIFERACIÓN | Median % | 0 | 0,2 | 0,1 | 0,002 |
| | Interquartile | | | | |
| | Range | 0-0,1 | 0,1-2,17 | 0,1-0,27 | |
| IL6 | Median pg/ml | 140,9 | 23.805,91 | 7017 | 0,002 |
| | Interquartile | | | | |
| | Range | 128,49-460,3 | 5.232,74-23.805,91 | 1.519,98-23.805,91 | |
| PROLIFERATION | Median % | 0 | 0,45 | 0,1 | 0,0001 |
| | Interquartile | | | | |
| | Range | 0-0,17 | 0,1-1,3 | 0-0,2 | |
| IL6 | Median pg/ml | 112,88 | 23.805,91 | 11899,6 | 0,002 |
| | Interquartile | | | | |
| | Range | 36,03-175,97 | 7.905,48-23.805,91 | 2.073,3-16.660,5 | |
| PROLIFERATION | Median % | 0 | 0,1 | 0,1 | 0,0001 |
| | Interquartile | | | | |
| | Range | 0 | 0,1-0,75 | 0-0,17 | |
| IL6 | Median pg/ml | 27,83 | 23.805,91 | 3.027,79 | 0,001 |
| | Interquartile | | | | |
| | Range | 15,81-30,76 | 4.479,4-23.805,91 | 899,21-4.262,96 | |

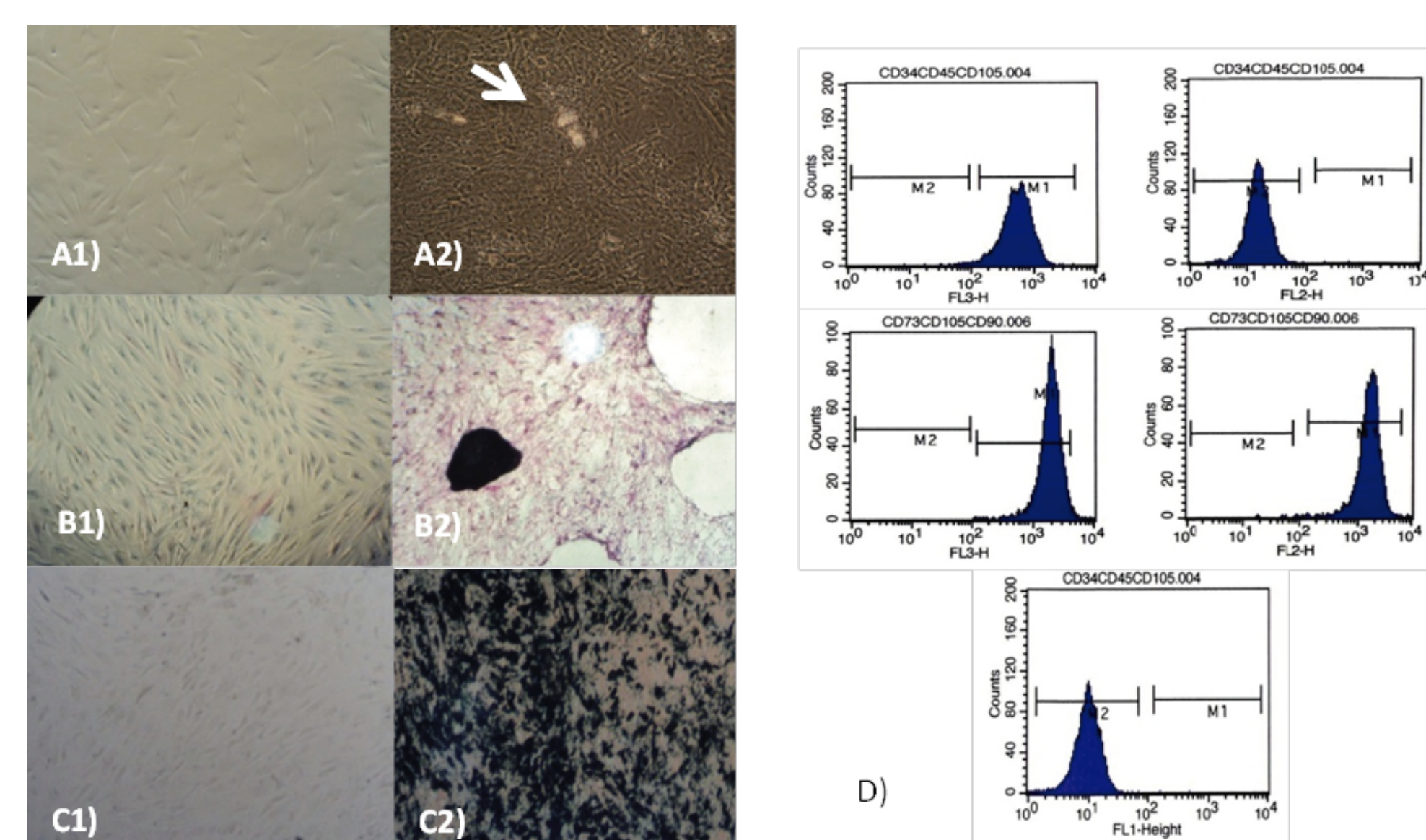


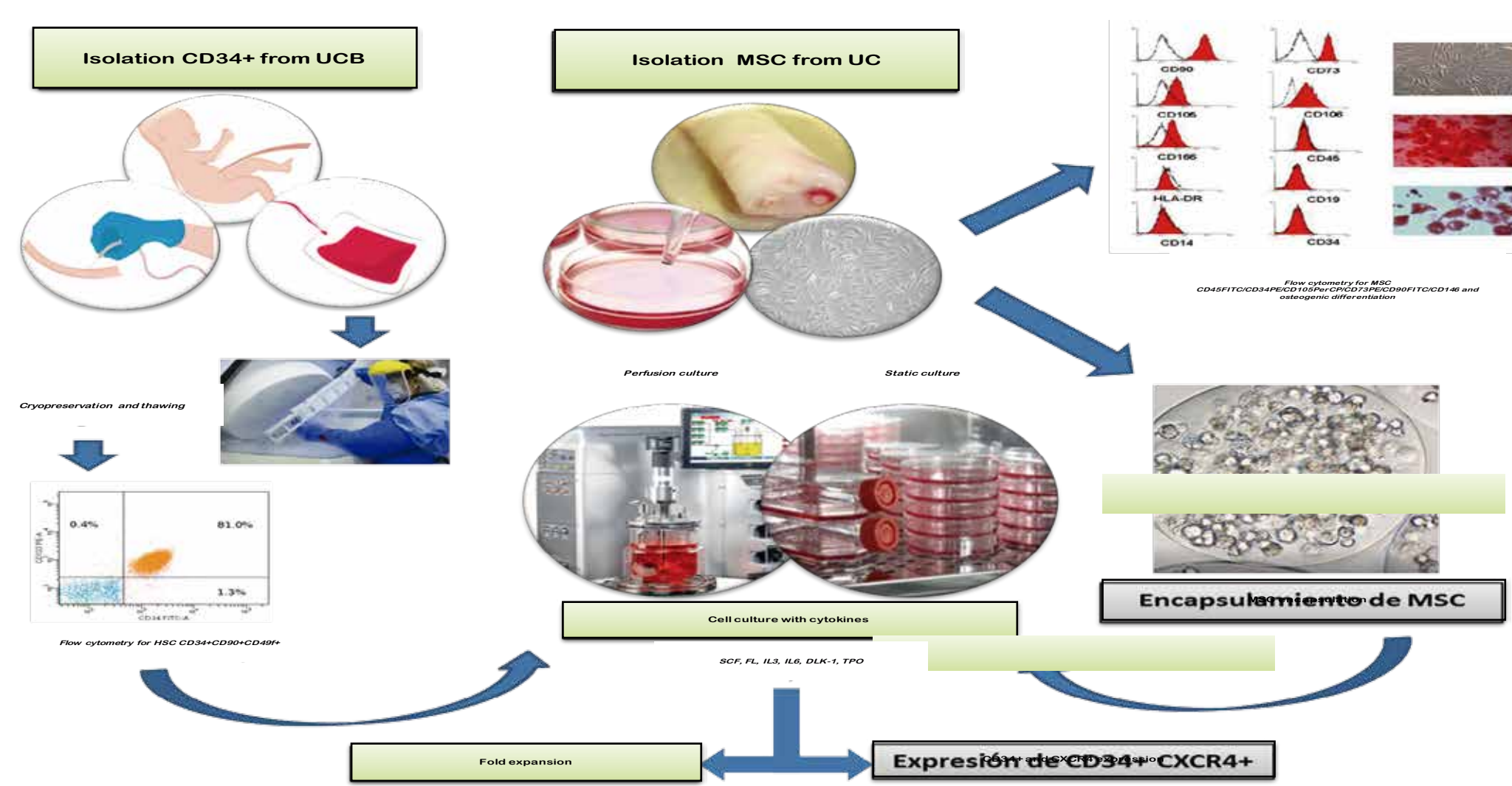
Figure 1. Morphology, potential of differentiation and phenotype of mesenchymal stem cells. A1) Day 12 of culture at 50% of confluence A2) Adipogenic differentiation after 21 days of cultivation. The arrow points to one of the vacuoles formed by the presence of fatty acids within the adipocyte . B) Osteogenic differentiation after 20 days of cultivation. B) Staining for alkaline phosphatase B1) differentiated cells B2) negative control. C) Von Kossa Staining C1) Differentiated cells C2) negative control. D) Flow cytometry MSC. Phenotype of one

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Introduction: Umbilical Cord Blood (UCB) has hematopoietic Stem cells (HSCs)z CD34+, these have the capacity for long term bone marrow repopulation, being easily obtained and criopreserved for their posterior use, which makes them a feasible alternative for transplant. The limited volume of obtained UCB, makes the count of HSCs to be low, having a repercussion on the probability of doing a graft on short term..

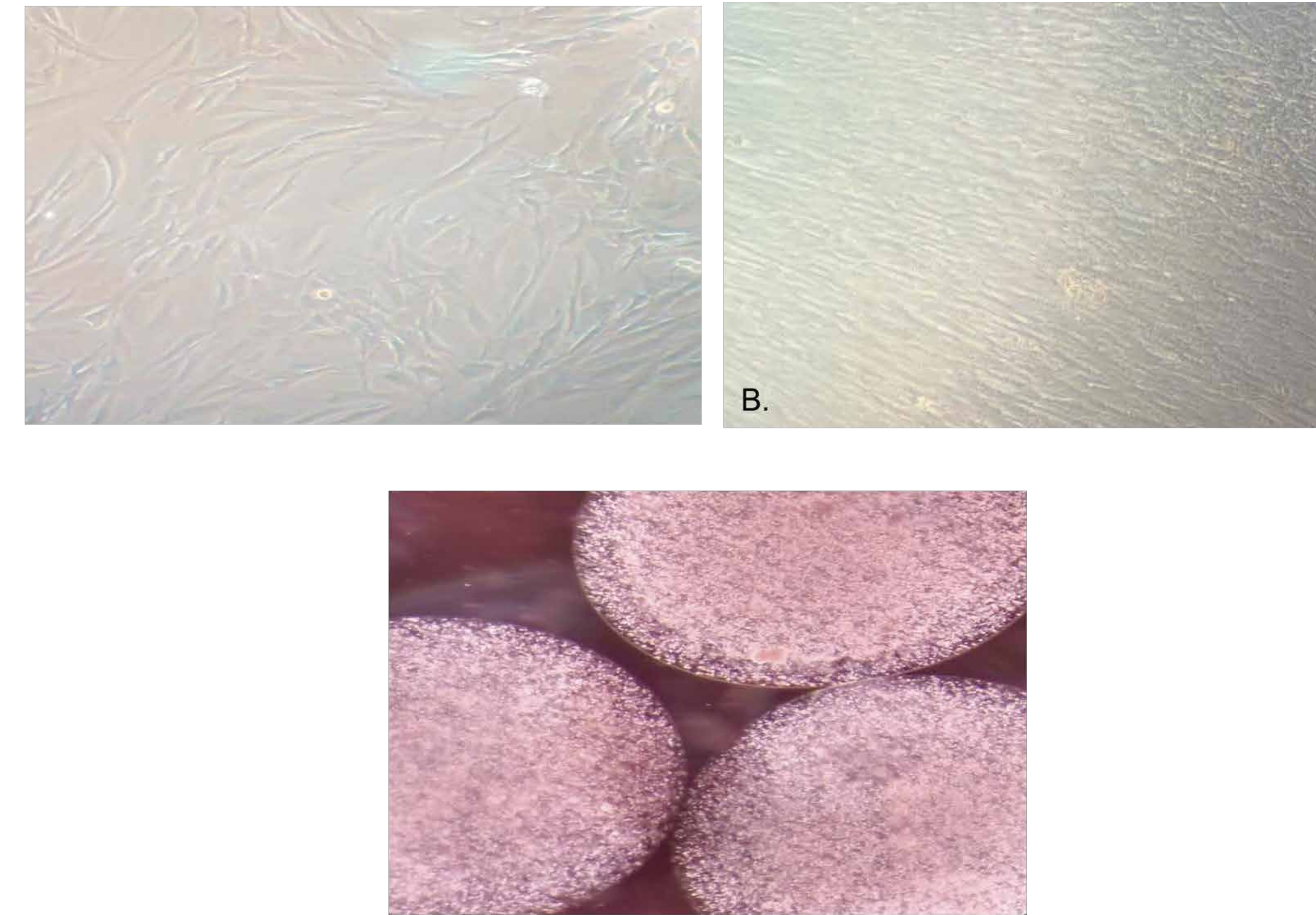
Our research is a comparison of two expansion methodos of CD34 +, CD49f +, CD90 + (static culture and bioreactor) using human recombinant cytokines (HRC), Stem cell factor (SCF), Interleukin 3 (IL-3), thrombopoietin (TPO) And ligand FT3 (FT3L) DLK-1 Interleukin 6 (IL6), umbilical cord Wharton CD146 + mesenchymal cells encapsulated in calcium alginate as mesenchymal stem cell feeder which compared to previously established models.

MATERIALS AND METHODS

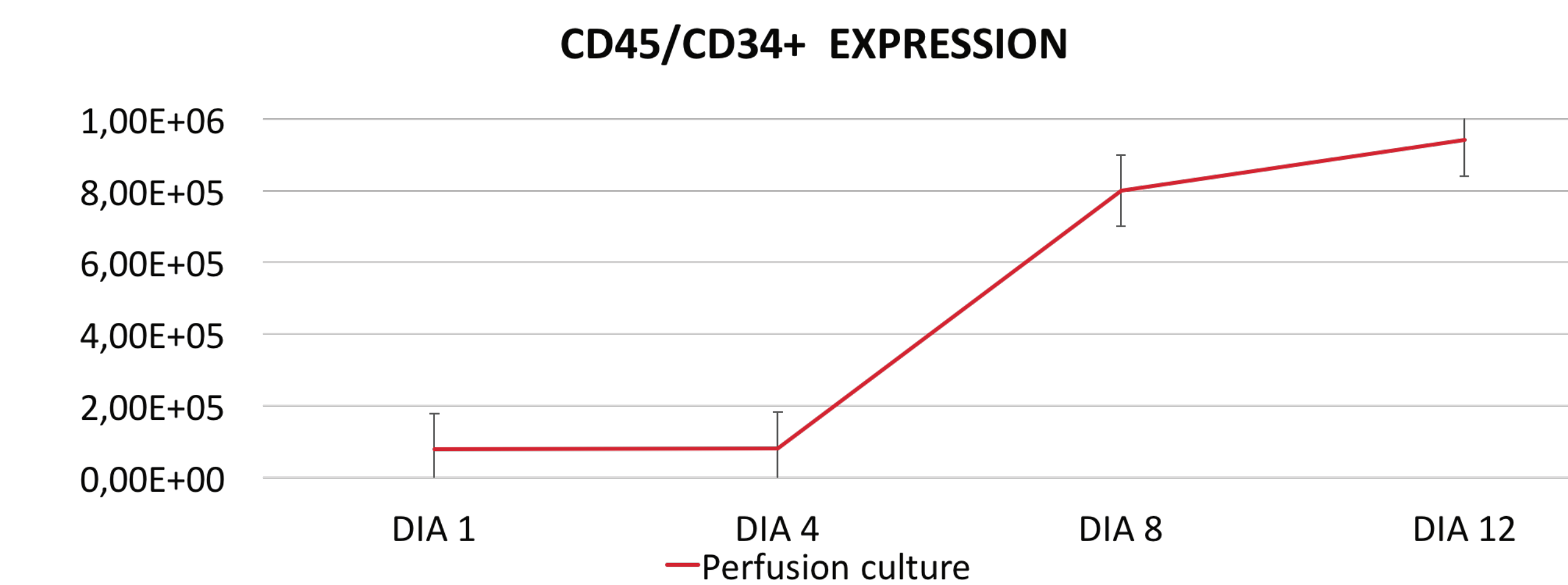
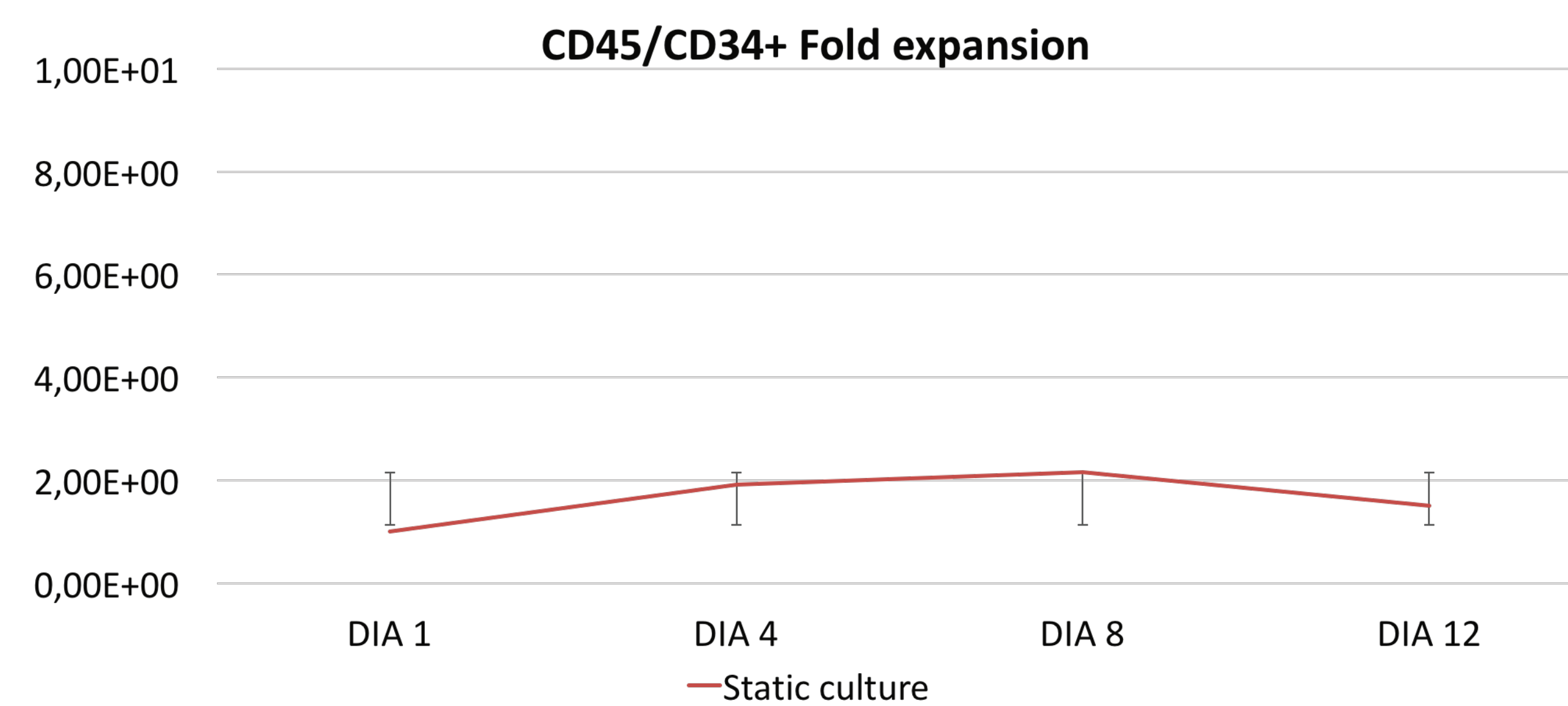


6 samples of UCB were taken prior to the signing of the informed consent of the parents. Red cell reduction was carried out on the nuclear cell concentrate and then cryopreserved for 1 year until thawed, CD34 + was selection by Kit Miltenyi. 11 umbilical cord samples were obtained to which nucleated cells were obtained by means of vein separation and digestion Wharton Jelly with type II collagenase. The mesenchymal cells were cultured and expanded, which were characterized by immunophenotype CD73 +, CD90 +, CD105 +, CD45 + and CD34 +. The potential for differentiation to osteogenesis was realized with the kit stem cells, the selection of the mesenchymal ones with the CD146 KIT was made and these were expanded.

RESULTS



Mesenchymal Stem cells from Wharton Jelly. A and B. MSC confluence 30% and 70%. C. MSC encapsulated in alginate.



CONCLUSIONS

- The model proposed (encapsulated mesenchymal stem cells from Wharton jelly and recombinant human cytokines), is possible increase ex vivo the number of CD34+ hematopoietic progenitor cells after 12 days of culture.
- This study evidenced a efficient model of HSC expansion in vivo, however this must be confirming for cultures to long term for confirm the trend shown, also assays in vivo with animal models.
- No significant differences in the expression of CXCR4 between CD34+ cells culture with and without mesenchymal cells, however this receptor showed a significative expression in the model proposed, showing that this cells are functional respect to movilization and nesting.

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