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Pooling umbilical cord-mesenchymal stromal cells derived from selected multiple donors reduces donor-dependent variability and improves their immunomodulatory properties

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Abstract

Background Umbilical Cord-derived Mesenchymal Stromal Cells (UC-MSCs) display high immunoregulatory properties, offering new perspectives to treat severe immune and inflammatory diseases. However, the heterogeneity of their biological properties remains a challenge to predict clinical response. The aim of our study is to evaluate a strategy based on the constitution of a pool of several pre-selected donors to reduce the biological variability of UC-MSCs and improve their immunomodulatory properties.

Methods Umbilical cords were collected from 10 healthy donors. Isolated UC-MSCs were characterized in the basal state and after a pro-inflammatory priming in vitro by interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα). Proliferation, immunophenotype, the expression of activation markers and the inhibition of T cell proliferation in vitro were assessed in UC-MSCs from selected single donors and from pools.

Results Our study highlights the donor-dependent heterogeneity of UC-MSCs immunomodulatory functions. Based on their ability to suppress T-cell proliferation in vitro, we classified donors into three profiles: high, medium and low. Preparation of pools containing UC-MSCs derived from each profile in a 1:1:1 ratio reduced the donor-dependent variability and, most importantly, improved the lowest immunomodulatory functions. After priming with pro-inflammatory cytokines, the inhibition of T-cell expansion by the pooled UC-MSCs was significantly higher than the low donor and the theoretical mean of individual donors, and was associated with increased expression of the key immunoregulatory proteins. Interestingly, the pool did not induce a cumulative immunogenic effect: expression of HLA or costimulatory molecules between the high donor and the pool were similar. Finally, pooling UC-MSCs derived from high and low donors in a 1:2 ratio was sufficient to enhance the lowest immunomodulatory properties.

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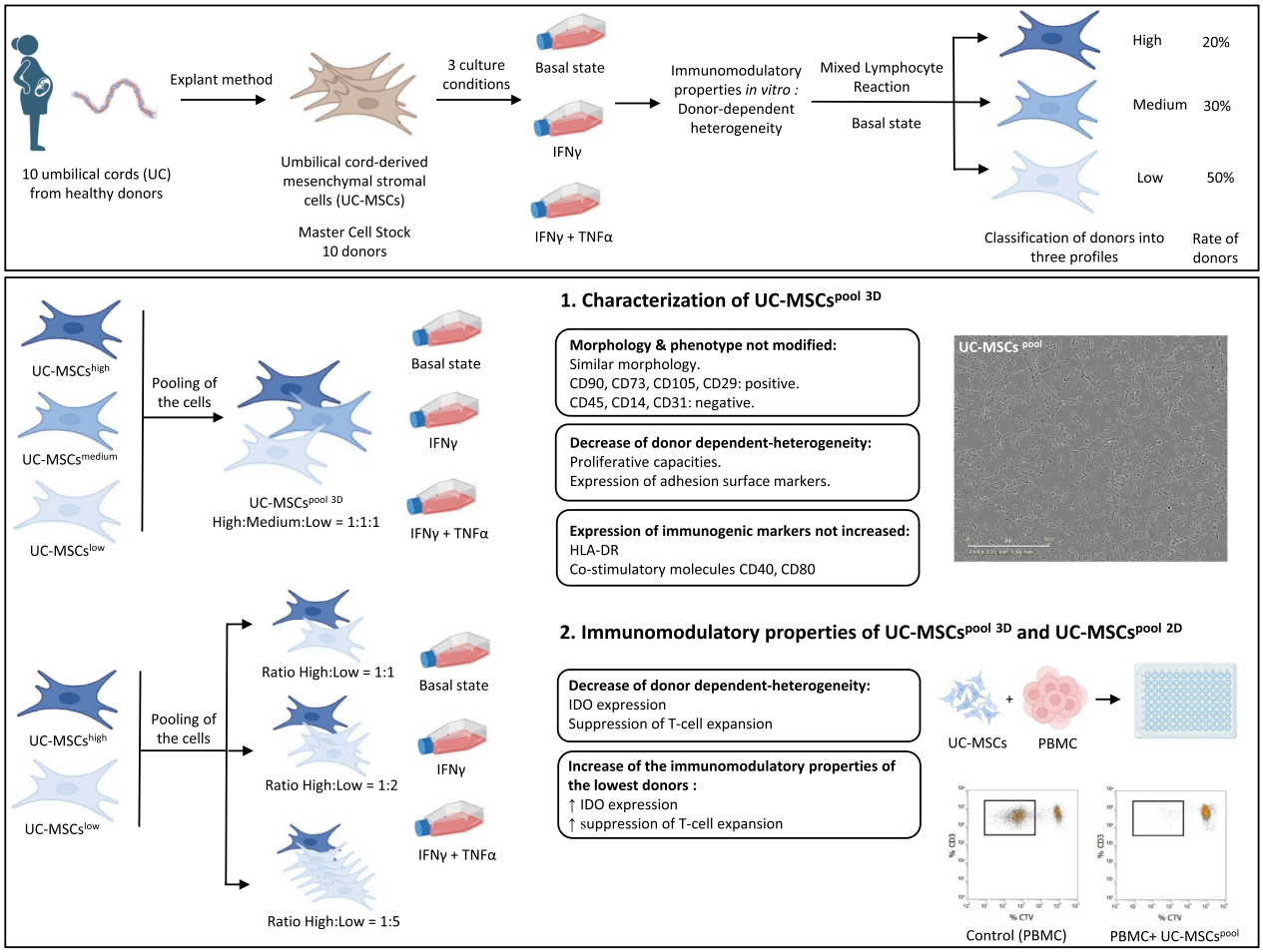
Conclusion Overall, our results demonstrate that pooled UC-MSCs with selected high donor offers a new strategy to optimize the immunomodulatory functions of allogeneic UC-MSC-based medicinal products.

Keywords Human umbilical cord, Wharton's jelly, Mesenchymal stromal cells, Immunomodulation, Donor heterogeneity, Donor variability, Pool

Graphical Abstract



Background

Mesenchymal stromal cells (MSCs) have been extensively studied for the development of advanced therapy medicinal products (ATMPs), in particular cell and tissue engineered therapies. However, only a few MSC-based cellular therapies have been approved, highlighting the complexity of transferring MSC-based products from bench to market. The main challenge is the high heterogeneity of the biological properties of MSCs, making it difficult to standardize production processes and to predict clinical responses. This variability may be related to culture conditions, cell doses, passages, or be donor-dependent or tissue-specific [1–6]. Umbilical cord (UC)-derived MSCs (UC-MSCs) also known as Wharton's jelly-derived MSCs display higher immunoregulatory properties than MSCs derived from adult tissues (bone marrow, adipose tissue), and therefore appear more interesting for the treatment of immune and inflammatory diseases [3, 7–10]. However, the donor-to-donor variability of UC-MSCs is greater compared with MSCs derived from adult tissues [8]. We previously reported that UC-MSCs, isolated from 12 different donors, exhibited a high variability of cell isolation yield, and significant differences in their proliferative capacities

[11]. Moreover, predicting the therapeutic effect of these cells is faintness due to the lack of predictive biomarkers to identify optimal donors. Currently, most MSC-based products under development use MSCs isolated from a single donor, with the risk of an insufficient clinical benefit if the cells have poor biological properties. Several strategies have been evaluated to enhance and normalize the immunomodulatory functions of MSCs including preconditioning with pro-inflammatory cytokines (interferon-γ, IFNγ and tumor necrosis factor-α, TNFα), vitamins (retinoic acid), or culture in a hypoxic environment [1, 5, 12, 13]. Transcriptomic analysis showed that cytokine treatment of MSCs did not reduce variations in biological properties and was associated with a decrease in cell cycle division [5]. In addition, an in vivo study in porcine model showed that stimulation of UC-MSCs with IFNγ can induce their immunogenicity, resulting in cells rejection or immunogenic reaction [14]. Hypoxic conditions resulted in preservation of the proliferative capacities of umbilical cord blood-derived MSCs without homogenizing their functions between donors, which underlies the heterogeneity of MSCs response to hypoxia [12]. In addition, MSC exposure to very low oxygen levels (0.1%) decreases the secretion of immunomodulatory cytokines [15].

More recently, pooling MSCs isolated from multiple donors has emerged as a new strategy to reduce donor-dependent heterogeneity [16, 17]. Few clinical trials have been reported, using bone marrow-derived MSCs in graft-versus-host disease (GvHD) and critical limb ischemia [18–20]. They suggest that pooled MSCs from multiple donors result in better clinical benefit compared with MSCs isolated from a single donor. Conversely, Hejretova et al. showed similar immunomodulatory properties in vitro between single donors and pooled MSCs [21]. These differences may be explained by current strategies which pool MSCs from random donors. Thus, prior selection of donors could improve the biological properties of pooled MSCs. For example, pooled UC-MSCs based on the baby's gender, have different proliferation capacities and cytokines expression profiles [22].

In this study, we aimed to evaluate a strategy based on the constitution of a pool of several pre-selected donors to reduce the biological variability of UC-MSCs and improve their immunomodulatory properties. We therefore assessed human UC-MSCs derived from 10 donors and confirmed the donor-to-donor heterogeneity of UC-MSC immunoregulatory functions. Then, we graded the donors into three profiles based on their immunoregulatory potential. Finally, we prepared pools including UC-MSCs isolated from at least one donor with a high potential, and assess the benefits on immunomodulatory properties.

Methods

UC-MSCs isolation and expansion

UCs were collected from 10 healthy donors, according to the French regulation. UC collection, and UC-MSC isolation and expansion were performed as previously described [11].

Preparation of UC-MSC pools

Cells isolated from single donors were thawed between passages 2 and 5 maximum based on our previous work [11], washed, enumerated then seeded at a density of 1000 cells/cm^2 for each donor, in 75 cm^2 culture flasks using a good manufacturing practice (GMP)-compliant complete culture medium composed of NutriStem® MSC XF Basal Medium (Biological Industries, Ref 05-200-1A) + Nutristem® MSC XF Supplement Mix (Biological Industries, Ref 05-200-1U) + 5% irradiated platelet lysate (PL) MultiPL100'i (Macopharma, Ref BC0190032) + sodium Heparin 2 IU/mL (Panpharma, Ref 5520508). UC-MSCs were harvested using a recombinant trypsin when they reached 80% of confluence. Harvested cells from each single donor were pooled in pools of three donors (3D) then used for analysis.

Cell morphology

UC-MSCs morphology was measured using the Incucyte® S3 and analyzed on the Incucyte® S3 Live—Cell Analysis system (Sartorius). The morphology was assessed in single donors and in the pool, in the basal state.

Cell counting

UC-MSCs from individual donors and from pools were enumerated after each passage using a manual Malassez counting Chamber. The Doubling Time (DT) was determined after each passage according to the formulae $(T \times \log(2))/(\log Y - \log X)$ (X: number of cells originally seeded, Y: final number of cells, T: duration of culture in hours).

Priming with pro-inflammatory cytokines

To assess their immunomodulatory properties, UC-MSCs were primed by IFNγ 10ng/mL and IFNγ + TNFα 10 + 10 ng/mL, for 48 h, based on ISCT recommendations and on our previous work [11, 23]. A non-treated (NT) condition was used to assess UC-MSCs in the basal state. After conditioning with cytokines, cells were harvested using a recombinant trypsin EDTA solution (Life Technologies, Ref 12563011), washed and

suspended in the appropriate medium depending on the analysis.

UC-MSC phenotype

The UC-MSC phenotype was assessed for each single donor then for the pool, in the basal state (NT) and after pro-inflammatory priming, as previously described [11]. Briefly, cells were suspended in 100 µL PBS/albumin 1% and stained concomitantly with several antibodies divided in two panels (additional file 1), for 15 min at 4 °C, protected from the light. Cells were washed in 1 mL PBS/Albumin 1%, centrifuged at 1500 RPM for 5 min and suspended in 300 µL PBS/albumin 1%. Negative controls were non-stained cells or Fluorescence Minus One for CD31, CD14 and CD45 antibodies. The acquisitions were performed with Attune NxT™ Thermofisher® Flow Cytometer and analyzes were performed using Attune NxT software.

Expression of activation markers

The expression of activation markers was evaluated on UC-MSCs of single donors then on pooled cells, in the basal state and after priming. The expression of indoleamine 2,3-dioxygenase (IDO), inter-cellular adhesion molecule-1 (ICAM-1/CD54), programmed death-ligand 1 (PD-L1/CD274), vascular cell adhesion molecule 1 (VCAM-1/CD106), melanoma cell adhesion molecule (MCAM/CD146), activated-leukocyte cell adhesion Molecule (ALCAM/CD166), CD44, CD200, IFNγ-receptor (IFNγ-R/CD119) and TNFα-receptor II (TNF-RII/CD120b) were assessed according to the previously described protocol (Additional file 1) [11]. Acquisitions were performed using Attune NxT™ Thermofisher® Flow Cytometer and analyses using Attune NxT™ software.

Mixed Lymphocyte Reaction (MLR)

MLR potency assay was performed on UC-MSCs both in the basal state and after pro-inflammatory conditioning for 48 h, according to Nicotra et al. [24]. Peripheral blood mononuclear cells (PBMC) pooled from 10 healthy donors were co-cultured with UC-MSCs from each donor or with UC-MSCs pooled from three donors. Ratios of UC-MSCs/PBMC 0/1 (control), 1/10, 1/30, 1/100 and 1/300 were used.

Statistical analysis

Statistical analyses were performed using GraphPad PRISM® 10.1.2 software with appropriate tests after assessment for non-normal distribution. Descriptive data are expressed as median [min—max] and all other values are expressed as mean ± standard deviation. A minimum of 95% confidence interval was established for significance. A p-value < 0.05 was considered statistically significant and are indicated on the figures as *: < 0.05, **: < 0.01, ***: < 0.001, ****: < 0.0001. Dunn's multiple comparison test was used to compare 10 donors, the Spearman test was used for correlation analysis, the Mann–Whitney test was used for comparison between two groups and the Tukey's test was used for multiple comparison of more than two groups.

Results

Characteristics of collected UCs

UCs were collected from 10 healthy mothers aged from 27 to 37 years (Table 1). The median of delivery term was 40.1 weeks (W) [39–41 W], the delivery was vaginal for 9/10 and cesarean for 1/10. Four of the newborns were female and six male (F/M = 0.67), with a median weight of 3480 g [2910–4200 g]. The median weight of the UCs was 22.0 g [15–43 g]; nine of them were manipulated within 1 day and one within 3 days.

Table 1 Characteristics of collected UCs (n = 10 donors)

UC	Mother age (y)	Delivery term (w)	Delivery	Child gender	Child weight (g)	UC weight (g)	Procurement (d)
UC 179	27	40.3	Vaginal	F	4200	24	1
UC 180	33	39.6	Cesarean	F	NA	16	3
UC 184	37	39.4	Vaginal	F	NA	25	1
UC 185	34	40.7	Vaginal	M	NA	15	1
UC 186	37	39.0	Vaginal	M	3930	43	1
UC 187	36	40.7	Vaginal	M	3480	36	1
UC 189	35	41.0	Vaginal	M	2910	16	1
UC 190	27	41.0	Vaginal	M	3420	15	1
UC 191	30	39.8	Vaginal	M	3290	26	1
UC 5A	29	39.0	Vaginal	F	3620	20	1

NA not available, *g* grams, *d* days, *w* weeks, *y* years

Isolated cells exhibited the morphological and phenotypic characteristics of UC-MSCs as previously described [11]. The total number of isolated cells at passage 0 (P0) was not correlated with the weight of the UCs (Additional file 2). The number of isolated UC-MSCs per gram of UC was not different depending on the gender of the babies, or on the delivery method (Additional file 2).

UC-MSCs immunomodulatory functions are donor-dependent

We analyzed the immunomodulatory properties of the UC-MSCs derived from each individual donor. To limit the use of our master stocks (MCS), descriptive analysis were performed on 10 donors with n = 1 independent experience per donor. First, we assessed the expression of IDO, a key protein involved in MSC immunomodulatory function, in the basal state (non-treated, NT) and in pro-inflammatory conditions (Fig. 1A, Additional file 3). IDO expression was low in UC-MSCs in the basal state (0.92% [0.04–18.68]) and was induced after priming with IFNγ (69.28% [1.28–87.38], p = 0.0022) and IFNγ + TNFα (67.22% [2.01–92.48], p = 0.0017). Even after pro-inflammatory priming, we observed an important heterogeneity between donors, with IDO expression > 60% for seven donors and ≤ 30% for three, including one donor with an IDO expression ≤ 2%.

To confirm this variability and identify the best UCs to use in clinic; we performed an MLR potency assay that has been previously validated in our laboratory as a GMP-compliant quality control for UC-MSC-based ATMPs [24]. To reproduce the clinical qualification of an ATMP, we performed MLR assays on UC-MSCs in the basal state. The median inhibition of T-cell proliferation with a ratio of 1/30 was 53.1% [35.5–96.1%] and this was used as a specification to validate the conformity of the MLR assay (Fig. 1B, Additional file 4). Based on this parameter, five UCs were compliant (> 53.1%) including UC185 (96.1%), UC180 (94.7%), UC190 (76.7%), UC191 (59.5%) and UC189 (55.0%), and five were out-of-specifications (OOS) (< 53.1% corresponding to our specification) including UC186 (51.1%), UC5A (49.3%), UC184 (42.1%), UC179 (36.5%) and UC187 (35.5%). We then used the interquartile range to distinguish two profiles within the compliant products: UC185 and UC180 displayed a high capacity (> 81.2%) to inhibit T-cell proliferation whereas UC190, UC191 and UC189 presented a medium inhibitory function. All OOS products were classified as UCs with low immunomodulatory potential.

Of note, neither the IDO expression nor the suppression of T-cell proliferation were associated with mother's age, delivery term, baby's gender and weight, or UC weight (Additional file 5).

Based on these data, we used the MLR functional assay to distinguish three profiles of UC-MSC donors with high, medium, or low immunosuppressive potential.

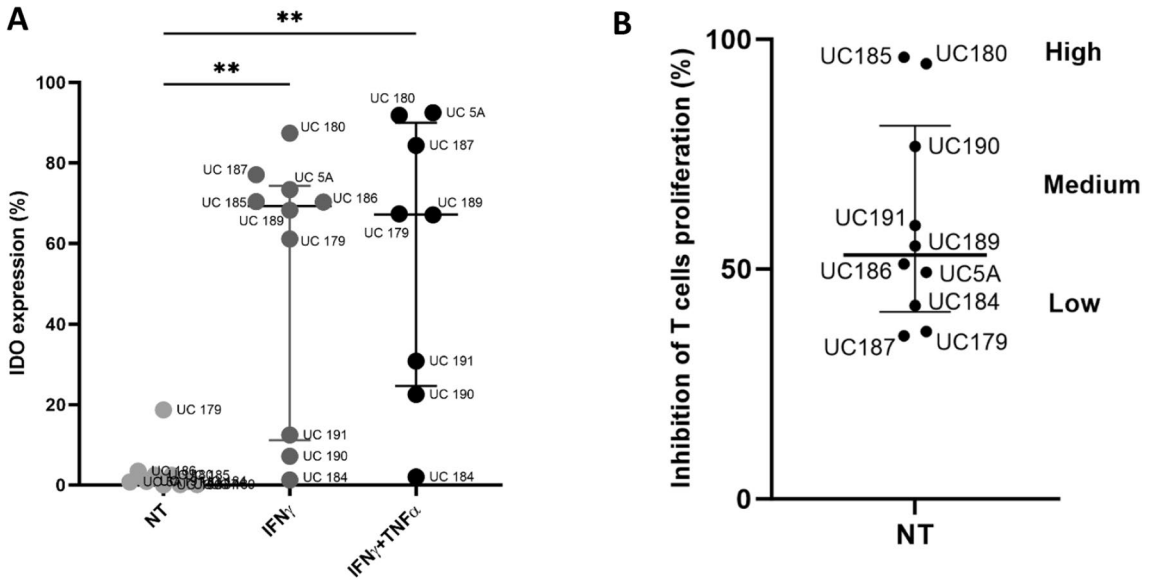


Fig. 1 Description of immunomodulatory properties of each of the 10 donors. **A** Median (left graph) and individual (right graph) expression of IDO (%) in the basal state (NT) and after pro-inflammatory priming by IFNγ and IFNγ + TNFα (n = 10 for NT and IFNγ, n = 8 for IFNγ + TNFα, UC185 and UC186 were not treated with IFNγ + TNFα). **B** Inhibition of T-lymphocyte proliferation (%) assessed by MLR assay in the basal state (NT), at UC-MSCs:PBMC ratio = 1:30 (n = 10 donors)

Constitution of UC-MSC pool

To better assess the donor-dependent variability of the biological properties of the UC-MSCs, we analyzed UC-MSCs isolated from UCs of each donor profile (minimum n = 3/donor): high (UC180), medium (UC190) and low (UC184) that we called UC-MSCs^{high}, UC-MSCs^{medium}, and UC-MSCs^{low} respectively. We then compared the properties of the UC-MSCs from individual donors to a pool of UC-MSCs from these three donors (UC-MSCs^{pool}). The UC-MSCs^{pool} consisted of UC-MSCs^{high}, UC-MSCs^{medium}, and UC-MSCs^{low} at a ratio of 1:1:1 (Fig. 2A).

No change in cell morphology was observed in the pool (Fig. 2B). The UC-MSCs both from individual donors of each profile (UC-MSCs^{high}, UC-MSCs^{medium}, and UC-MSCs^{low}) and from the pool expressed mesenchymal surface markers (CD90, CD73, CD105, CD29) but not hematological or endothelial markers (CD45, CD14, CD31) in the basal state (Additional file 6).

Pooling UC-MSCs reduces the inter-donor variability of proliferative capacities

As the manufacturing of cell-based therapies is time and cost consuming, MSCs proliferative potential in vitro is a critical parameter for donor selection. We assessed the doubling time (DT) (h) between passages 3 and 4. In the basal state, the DT of UC-MSCs^{low} (42.5 ± 11.8 h) was slower than of other donors (UC-MSCs^{high}: 31.5 ± 15.5 h, p > 0.05; UC-MSCs^{medium}: 25.2 ± 3.6 h, p = 0.0126) as well as UC-MSCs^{pool} (27.8 ± 6.6 h, p = 0.0362) (Fig. 2C). As the production process may include MSCs activation with cytokines, we also evaluated their expansion after stimulation with IFNγ and IFNγ + TNFα. After priming with IFNγ, the DT of UC-MSCs^{low} (42.8 ± 17.1 h) was still significantly slower than of UC-MSCs^{medium} (26.1 ± 3.2 h, p = 0.0165) and UC-MSCs^{pool} (27.2 ± 6.2 h, p = 0.0234). Finally, treatment with IFNγ + TNFα showed lower proliferation of UC-MSCs^{low} (49.6 ± 18.7 h) compared with UC-MSCs^{high} (32.3 ± 9.7 h, p = 0.0163), UC-MSCs^{medium} (30.4 ± 4.6 h, p = 0.0043) and UC-MSCs^{pool} (33.0 ± 11.3 h, p = 0.0139) (Fig. 2C). Interestingly, the theoretical mean of the DT of individual donors was similar to the DT of pooled UC-MSCs in non-treated and treated conditions (Fig. 2C). Taken together, these results showed that pooling UC-MSCs reduces the donor-dependent heterogeneity of the proliferation capacities.

Pooling UC-MSCs results in homogenization of the expression of adhesion surface markers

Despite the growing interest of the paracrine properties of MSCs and the use of their extracellular vesicles,

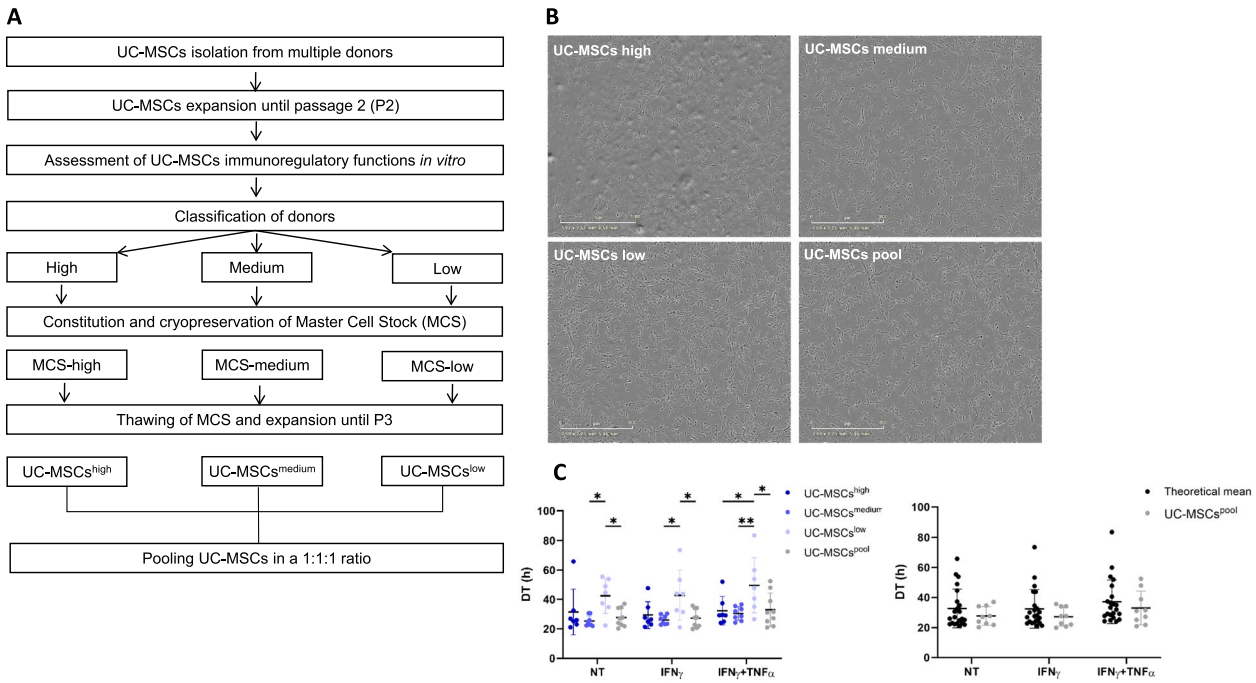


Fig. 2 Constitution of UC-MSCs pools with prior selection of donors based on their immunoregulatory functions. **A** The process of constitution of UC-MSC pools. **B** Morphology (4 × magnification) of UC-MSCs^{high}, UC-MSCs^{medium}, UC-MSCs^{low} and UC-MSCs^{pool}. **C** Comparison of the doubling time (DT) between individual donors (n = 7 independent experiences per donor and per condition) and the pool (n = 9 independent experiences per condition) (left graph) and the theoretical mean of individual donors and the pool (right graph)

cell-to-cell interaction remains a key parameter to guarantee maximum efficacy of the immunosuppressive functions of MSCs [25, 26]. Thus, we assessed the expression of several markers described to be involved in MSC cell-to-cell direct mechanisms. In the basal state UC-MSCs from each donor and from the pool expressed several adhesion molecules including CD44, ALCAM/CD166, MCAM/CD146, ICAM-1/CD54, and CD200.

The expression of CD44 was similar between individual donors and compared with pooled UC-MSCs both in the basal state and after pro-inflammatory treatment (Fig. 3A). ALCAM/CD166 showed donor-dependent heterogeneity in UC-MSCs in the basal state with increased expression in UC-MSCs^{medium} (83.1 ± 18.6%) compared with UC-MSCs^{high} (32.1 ± 30.8%, $p = 0.0253$) and UC-MSCs^{low} (43.9 ± 8.5%, $p > 005$) (Fig. 3B). Pooling the cells resulted in an increased expression of ALCAM/CD166 (81.1 ± 7.1%) that became similar with UC-MSCs^{medium} and higher than UC-MSCs^{high} ($p = 0.0186$) and UC-MSCs^{low} ($p > 0.05$). In pro-inflammatory conditions, the expression of ALCAM/CD166 was still donor-dependent and was increased in pooled UC-MSCs (Fig. 3B). Similar results were observed with MCAM/CD146, with increased expression after pooling UC-MSCs in the basal state as well as in pro-inflammatory conditions (Fig. 3C). The expression of ICAM-1/CD54 in the basal state was higher in UC-MSCs^{high} (85.1 ± 13.5%) than in UC-MSCs^{medium} (28.4 ± 17.5%, $p = 0.0004$) and UC-MSCs^{low} (59.3 ± 44.7%, $p > 0.05$). Pooling the cells did not increase the lowest expression of ICAM-1/CD54, whereas pro-inflammatory treatment increased the expression to > 95% in individual donors and the pool (Fig. 3D). The expression of CD200 was also higher in UC-MSCs^{high} in the basal state and remained similar after pro-inflammatory treatment. Pooling the UC-MSCs increased CD200 expression compared with UC-MSCs^{medium} and UC-MSCs^{low} but was not significant (Fig. 3E).

VCAM-1/CD106 and PD-L1 were not expressed in the basal state but were stimulated after pro-inflammatory treatment (Fig. 2F–G). After treatment with IFNγ + TNFα, VCAM-1/CD106 was highly expressed in UC-MSCs^{medium} whereas PD-L1 increased in UC-MSCs^{high} and UC-MSCs^{low}. Pooling the UC-MSCs resulted in an increase in the lowest expression of VCAM-1/CD106 and PD-L1 but was still not significant compared with each individual donor.

Taken together, these results highlighted the existence of an important donor-to-donor variability of the expression of adhesion surface markers, which tends to be standardized by pooling UC-MSCs.

The low immunogenicity of UC-MSCs is not modified in the pooled product

Neither the UC-MSCs of individual donors nor of the pool expressed HLA-DR in the basal state. After IFNγ priming, HLA-DR expression was strongly increased in UC-MSCs^{high} compared with UC-MSCs^{medium} and UC-MSCs^{low} (26.3 ± 11.1% vs 2.4 ± 1.7% vs 1.9 ± 1.6%, $p < 0.0001$) (Fig. 4A). Similar results were observed with IFNγ + TNFα (13.9 ± 5.0% vs 0.8 ± 0.7% vs 1.0 ± 0.9%, $p < 0.01$) (Fig. 4A). Interestingly, after IFNγ conditioning, HLA-DR expression was higher in UC-MSCs^{pool} than in UC-MSCs^{medium} and UC-MSCs^{low} (12.4 ± 4.1% vs 2.4 ± 1.7% vs 1.9 ± 1.6%, $p < 0.05$), but was still lower than in UC-MSCs^{high} (12.4 ± 4.1% vs 26.3 ± 11.1%, $p < 0.01$)

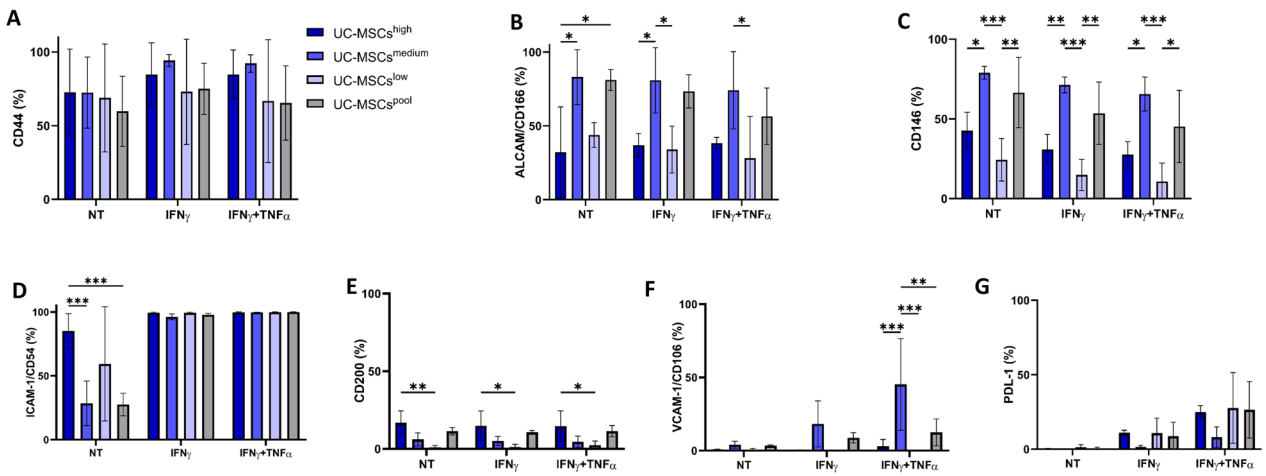


Fig. 3 Expression of adhesion surface markers in UC-MSCs isolated from individual donors versus pool. Expression (%) of **A** CD44, **B** ALCAM/CD166, **C** CD146, **D** ICAM-1/CD54, **E** CD200, **F** VCAM-1/CD106 and **G** PDL-1 in the basal state (non-treated) and after pro-inflammatory treatment with IFNγ and IFNγ + TNFα (n = 3 independent experiences per donor, pool, and per condition)

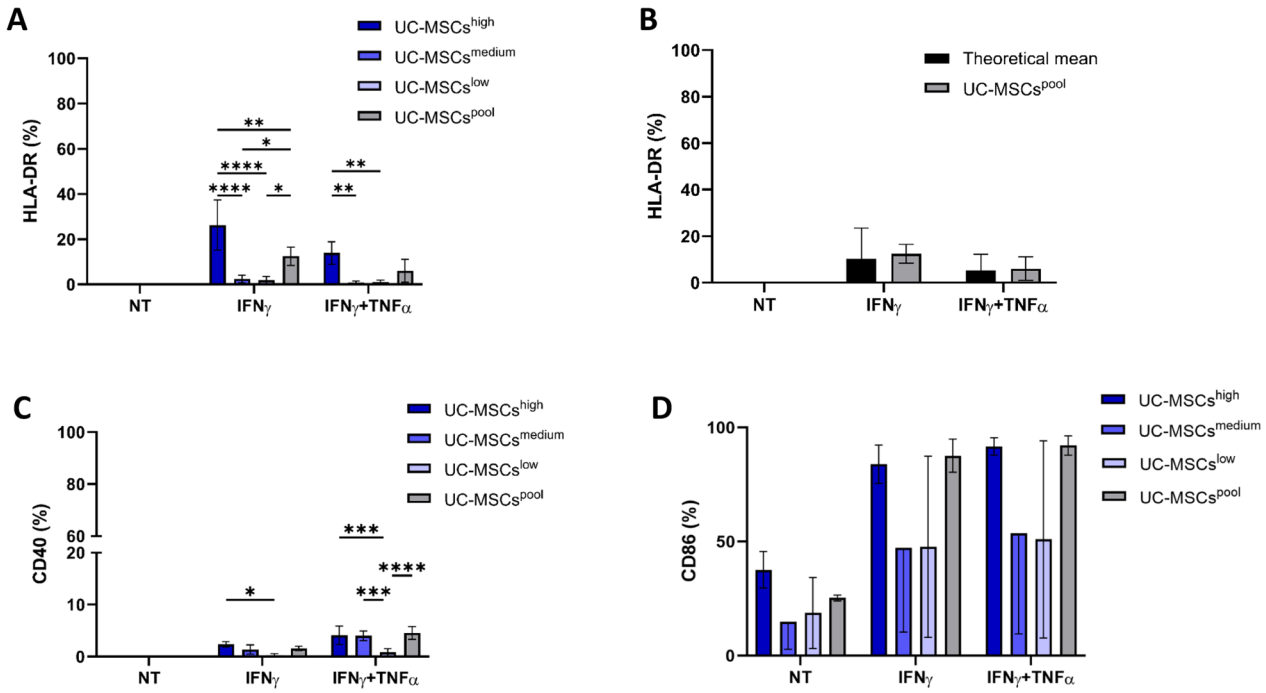


Fig. 4 Expression of immunogenicity markers in UC-MSCs isolated from individual donors versus pool. **A** Expression of HLA-DR (%) in individual donors versus pool. **B** Comparison of the theoretical mean of HLA-DR expression versus in the pool. Expression (%) of **C** CD40 and **D** CD86 in individual donors versus pool. UC-MSCs were assessed in the basal state (non-treated) and after pro-inflammatory treatment with IFNγ and IFNγ + TNFα (n = 3 independent experiences per donor, pool, and per condition)

(Fig. 4A). The expression of HLA-DR by UC-MSCs^{pool} was similar to the theoretical mean of individual donors after treatment with IFNγ (12.4 ± 4.1% vs 10.18 ± 13.3%) and IFNγ + TNFα (6.0 ± 5.1% vs 5.2 ± 7.0%) (Fig. 4B). In parallel, the expression of CD40 and CD86 co-stimulatory molecules was also heterogeneous between donors but presented similar profiles between UC-MSCs^{pool} and UC-MSCs^{high} (Fig. 4C and D). These results demonstrate that UC-MSCs pooled from multiple donors do not induce a cumulative immunogenic effect, which is encouraging for the clinical use of pooled UC-MSC-based ATMPs as an allogeneic product.

IFNγ-stimulated and pooled UC-MSCs enhance the lowest expression of IDO

IDO was not expressed in the basal state but was enhanced in pro-inflammatory conditions, with donor-dependent variability (Fig. 5A, Additional file 3). After treatment with IFNγ and IFNγ + TNFα, IDO expression was significantly higher in UC-MSCs^{high} than in UC-MSCs^{medium} and UC-MSCs^{low} (IFNγ: 75.1 ± 12.1% *vs* 18.5 ± 13.4% *vs* 10.1 ± 8.7%; p = 0.0003 and p < 0.0001, respectively; IFNγ + TNFα: 83.5 ± 9.0% vs 39.8 ± 26.4% vs 13.0 ± 9.7%; p = 0.0048 and p < 0.0001, respectively; Fig. 5A). IDO expression was significantly higher in

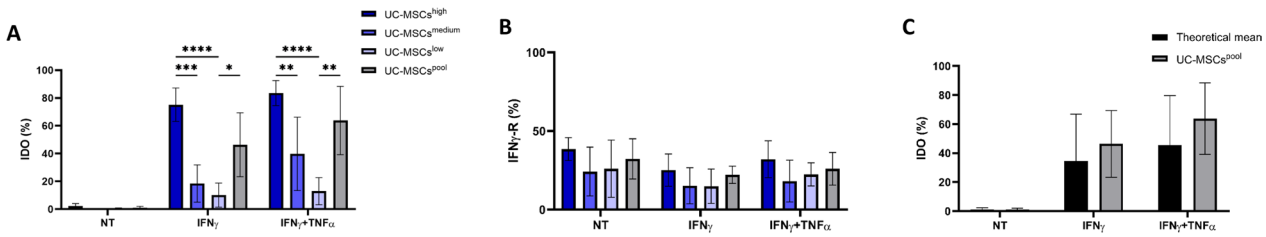


Fig. 5 Expression of IDO and IFNγ-Receptor in UC-MSCs isolated from individual donors versus pool. **A** IDO expression (%) in individual donors versus pool. **B** IFNγ-Receptor expression (%) in individual donors versus pool. **C** Comparison of the theoretical mean of IDO in individual donors versus in the pool. UC-MSCs were assessed in the basal state and after pro-inflammatory treatment with IFNγ and IFNγ + TNFα. (n = 3 independent experiences per donor, pool, and per condition)

UC-MSCs^{pool} compared with UC-MSCs^{low} (IFNγ: 46.4 ± 23.0% vs 10.1 ± 8.7%, p = 0.0217; IFNγ + TNFα: 63.8 ± 24.6% vs 13.0 ± 9.7; p = 0.001; Fig. 5A). Interestingly, these differences were observed despite the uniform expression of IFNγ-receptor (CD119) in each donor, as well as in pooled UC-MSCs (Fig. 5B). Moreover, the IDO expression by UC-MSCs^{pool} was similar to the theoretical mean of IDO expression by the three donors, after treatment with IFNγ (46.4 ± 23.0 vs 34.6 ± 32.3%) and IFNγ + TNFα (63.8 ± 24.6% vs 45.4 ± 34.2%) suggesting that pooling UC-MSCs results in average IDO expression thus reducing inter-donor variability (Fig. 5C).

Pooling UC-MSCs increases their potential to inhibit T-cell proliferation

In our previous work, we used a MLR potency assay [24] to demonstrate that UC-MSCs inhibit T-cell proliferation in a IFNγ-independent manner and that this inhibition increases after pro-inflammatory stimulation [11]. In this study, we confirmed that in the basal state, UC-MSCs from each single donor as well as from the pool, are able to suppress T-cell expansion in vitro in a dose-dependent manner (Fig. 6A, B, Additional file 4). The mean of T-cells suppression was > 50% in each single donor and in the pool when the UC-MSCs/PBMC ratio was 1/10. However, this activity was donor-dependent and more important in UC-MSCs^{high} than in UC-MSCs^{low} (82.9 ± 12.1% vs 51.1 ± 15.1%, p = 0.0278 for ratio UC-MSCs/PBMC = 1/10) in the basal state. After priming with IFNγ, T-cell suppression by UC-MSCs^{high} and UC-MSCs^{pool} became similar at a ratio of 1/10, and were both significantly higher than UC-MSCs^{medium} and UC-MSCs^{low} (Fig. 6B). Finally, after treatment with IFNγ + TNFα, the inhibition of T-cell expansion by UC-MSCs^{pool} at a ratio of 1/10 was still significantly higher than UC-MSCs^{low} (88.4 ± 10.2% vs 60.5 ± 7.6%, p = 0.0209). Interestingly, the inhibition of T-cell expansion by UC-MSCs^{pool} was significantly higher than the theoretical average of individual donors at a ratio of 1/10 after treatment with IFNγ (91.2 ± 5.4% vs 64.3 ± 16.9%, p = 0.0004) and IFNγ + TNFα (88.4 ± 10.2% vs 64.5 ± 15.8%, p = 0.026) (Fig. 6C).

Taken together, our results suggest that pro-inflammatory stimulation does not decrease the donor-dependent variability whereas the use of pooled UC-MSCs improves low immunomodulatory functions.

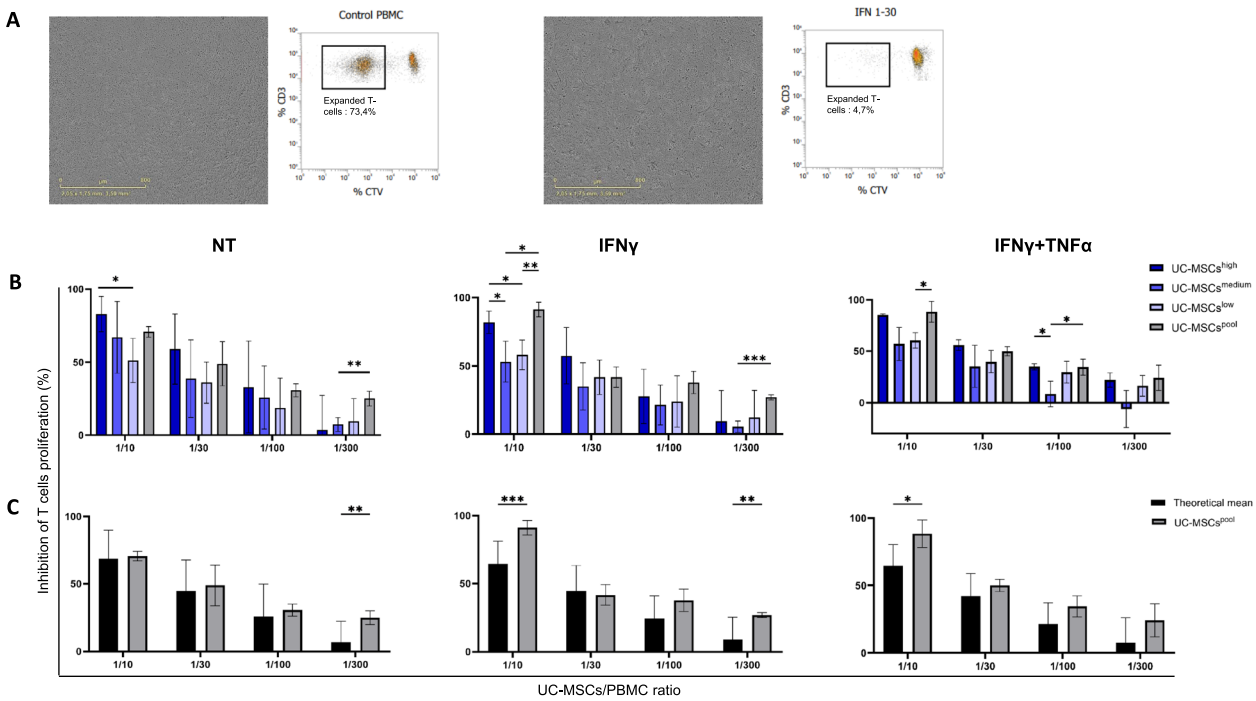


Fig. 6 Suppression of T-cells expansion by UC-MSCs in the basal state (NT) and after priming with IFNγ and IFNγ + TNFα. **A** Expanded T cells in the absence (ratio 0:1; left picture) and in the presence of UC-MSCs after priming with IFNγ (ratio 1:30; right graph). **B** Comparison of individual donors versus the pool with a decreased UC-MSCs:PBMC ratio of 1/10; 1/30; 1/100; 1/300. **C** Comparison of the theoretical mean of individual donors versus the pool. (n = 5 independent experiences per donor, pool, and per condition)

Pooling UC-MSCs from high and low donors with decreased quantity of UC-MSCs^{high} is sufficient to increase the lowest immunoregulatory functions

To optimize the constitution of pools and facilitate pharmaceutical manufacturing, which is time and cost consuming, we assessed the immunomodulatory properties of UC-MSCs pooled from two donors, high (UC185) and low (UC184), referred to as UC-MSCs$^{pool\ 2D}$. Regarding the rarity of UC-MSCs^{high} (20% in our study) we performed dose de-escalation of these cells in the pool 2D. Thus, UC-MSCs$^{pool\ 2D}$ consisted of UC-MSCs^{high} and UC-MSCs^{low} at ratios of 1:1, 1:2 and 1:5 (UC-MSCs$^{pool\ 2D\ 1:1}$; UC-MSCs$^{pool\ 2D\ 1:2}$; UC-MSCs$^{pool\ 2D\ 1:5}$ respectively). In the basal state, IDO was not expressed (< 2%) in UC-MSCs$^{pool\ 2D}$ regardless of the ratio. After treatment with IFNγ, IDO expression was significantly increased in UC-MSCs$^{pool\ 2D\ 1:1}$ compared with UC-MSCs^{low} (67.1 ± 14.5% versus 10.1 ± 8.7%, $p < 0.0001$) and remained higher with UC-MSCs$^{pool\ 2D\ 1:2}$ (47.5 ± 26.9%; $p = 0.0113$) and UC-MSCs$^{pool\ 2D\ 1:5}$ (47.0 ± 23.1%; $p = 0.0125$) (Fig. 7A). Priming with IFNγ + TNFα increased the difference between UC-MSCs$^{pool\ 2D}$ regardless of the ratio and UC-MSCs^{low}, while IDO expression in UC-MSCs$^{pool\ 2D}$ reached that observed in UC-MSCs^{high} (83.5 ± 9.0%) at a ratio of 1:1 (81.4 ± 8.5%) and remained high at a ratio of 1:2 (71.9 ± 14.4%) and 1:5 (65.0 ± 19.6%). Importantly, IDO expression in UC-MSCs pooled from two donors even at low ratios (1:2 and 1:5), was similar with the pool of three donors (Fig. 7B).

To confirm these results, we performed an MLR assay using the pool 2D at ratios 1:1 and 1:2 (UC-MSCs$^{pool\ 2D\ 1:1}$ and UC-MSCs$^{pool\ 2D\ 1:2}$). In the basal state, the suppression of T-cells expansion by UC-MSCs$^{pool\ 2D\ 1:1}$ tended to be better than UC-MSCs^{low} at a UC-MSCs/PBMC ratio = 1/10 (63.6 ± 27.1% versus 51.1 ± 15.1%, $p = 0.8777$) and became significantly higher at a ratio of 1/100 (68.5 ± 7.1% versus 18.7 ± 20.4%, $p = 0.0133$. Figure 7C). This suppression remained higher with UC-MSCs$^{pool\ 2D\ 1:2}$ compared with UC-MSCs^{low} (86.4 ± 8.1% versus 51.1 ± 15.1%, $p = 0.0194$ and 83.3 ± 10.8% versus 36.0 ± 14.1%, $p = 0.0094$ at an UC-MSCs/PBMC ratio = 1/10 and 1/30 respectively).

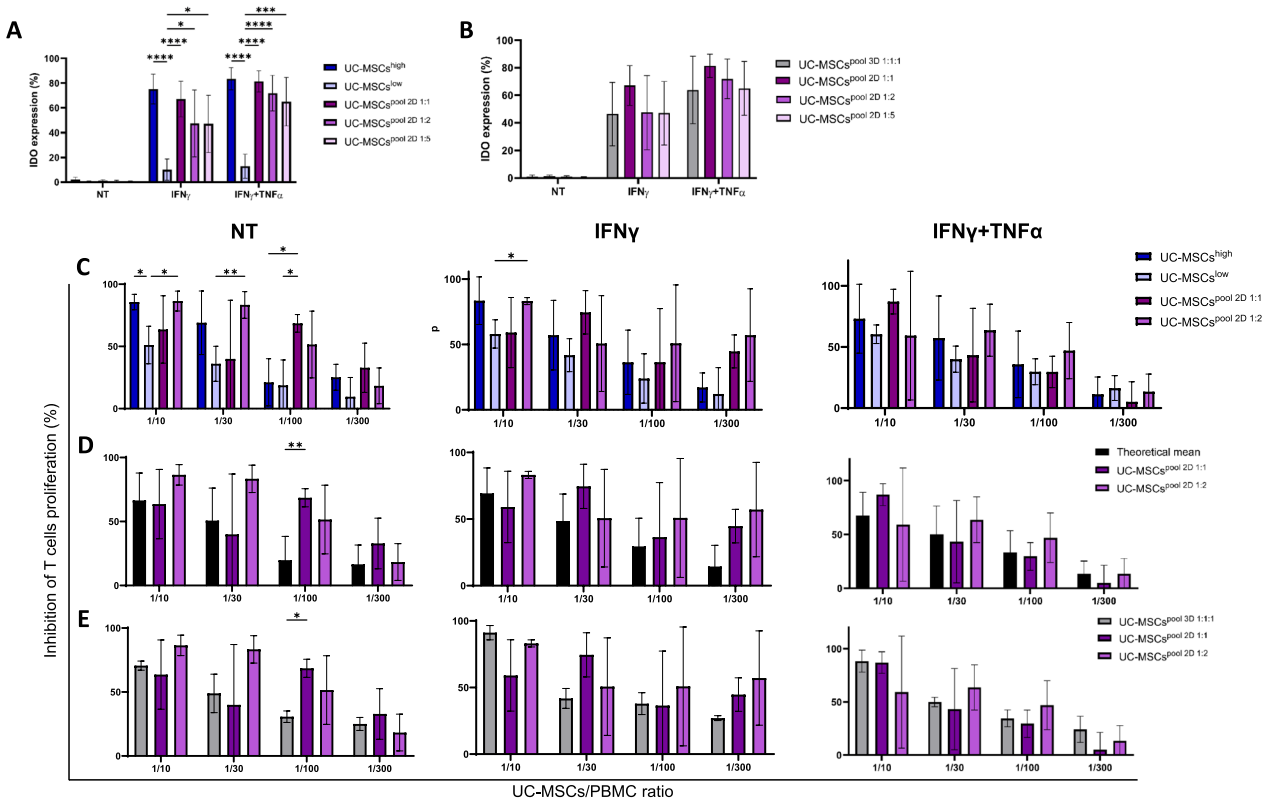


Fig. 7 Expression of IDO and suppression of T-cells by UC-MSCs isolated from high and low donors versus pool of two or three donors. **A** Expression (%) of IDO by cells from individual high and low donors and pools of these two donors (2D) at high:low ratio = 1:1; 1:2 and 1:5. **B** Comparison of IDO expression (%) between pools 2D and 3D. **C** Comparison of T-cell suppression between individual high and low donors versus the pool 2D with a decreased UC-MSCs:PBMC ratio of 1/10; 1/30; 1/100; 1/300. **D** Comparison of the theoretical mean of individual donors versus the pool 2D. **E** Comparison of T-cells suppression between pools 2D and 3D. (n = 5 independent experiences per donor and per condition and n = 3 independent experiences for pool 2D)

These results highlighted that pooling is still effective even with a decreased dose of UC-MSCs^{high}. After priming with IFNγ, T-cell suppression by UC-MSCs$^{pool\ 2D\ 1:2}$ was still higher than UC-MSCs^{low} (83.1 ± 2.7% versus 58.0 ± 10.8%, p = 0.0176 at an UC-MSCs/PBMC ratio = 1/10, Fig. 7C). Interestingly, the capacity of UC-MSCs$^{pool\ 2D}$ to suppress T cells was at least similar or even better than the theoretical mean of the two individual high and low donors (Fig. 7D). Finally, the comparison of the 3D pool with the 2D pools at ratios of 1:1 and 1:2 suggested a similar capacity to suppress T-cell expansion or even a higher T-cell suppression with the pool 2D 1:1 (68.5 ± 7.1% versus 30.7 ± 4.5%, p = 0.0422 in the basal state and with ratio UC-MSCs/PBMC = 1/100, Fig. 7E).

Discussion

MSCs derived from fetal tissues and in particular from the Wharton's jelly of UCs, are emerging as a promising perspective to develop cellular immunotherapies for the treatment of immune and inflammatory diseases. Compared with MSCs derived from adult tissues, UC-MSCs are highly proliferative and immunosuppressive due to their primitive status. However, the donor-dependent heterogeneity of UC-MSCs is greater than that of MSCs derived from adult tissues, which is a real challenge to develop standardized UC-MSC-based medicinal products and to predict the clinical response [8]. In this study, we confirmed the donor-to-donor heterogeneity of the UC-MSCs immunoregulatory functions. The expression of IDO was increased after priming cells with IFNγ and IFNγ + TNFα, but in a donor-dependent manner. The suppression of T-cells proliferation by UC-MSCs confirmed donor-dependent differences in their immunoregulatory properties in the basal state (without pro-inflammatory treatment). Our results demonstrated that the immunosuppressive ability of UC-MSCs is innate, regardless of the donor, as shown in our previous study [11], in contrast to adult tissues-derived MSCs which require pro-inflammatory induction [27].

Several studies have suggested that the donor's gender could be a variability factor of the biological properties of the MSCs [28, 29] while others suggest that the donor's age may have an impact [30]. In our study, we did not observe differences in IDO expression or suppression of T-cell proliferation according to the age of the mother, the term of delivery, the gender and weight of the baby, or the weight of the UCs. This may be explained by the primitive nature of UC-MSCs compared to MSCs derived from adult tissues. Therefore, the clinical characteristics of mothers or babies cannot be used as predictive factors. However, we could not evaluate the influence of the type of delivery or the time between cord collection and MSC isolation because only one donor (UC180) exhibited these particular characteristics. Thus, we cannot exclude the possibility that this has an impact on the biological activity of the UC-MSCs and it would be necessary to evaluate this with other donors.

Several strategies have been used to reduce this biological variability and develop MSC-based medicinal products with reproducible immunomodulatory functions. Zhang et al., indicated that inter-donor variabilities of UC-MSCs immunosuppressive properties can be eliminated after treatment with two pro-inflammatory cytokines, IFNγ and TNFα [5]. However, in our study we demonstrated the persistence of variability in immunoregulatory properties between donors after treatment with IFNγ and IFNγ + TNFα. Indeed, high IDO expression (> 60%) was only found in two thirds of the donors, while one third had low IDO expression (≤ 30%). The MLR potency assay corroborated this heterogeneity, with the persistence of three distinct profiles of immunomodulatory potential, even after pro-inflammatory stimulation. Thus, our results demonstrated that treatment with cytokines is insufficient to eradicate the heterogeneity and enhance the lowest immunomodulatory properties of UC-MSCs. In addition, because it has been shown to reduce MSC proliferation, treatment with cytokines during clinical manufacturing could cause problems [5].

Pooling MSCs from multiple donors is emerging as a promising strategy to reduce the donor-to-donor heterogeneity. Previous studies have already shown that pooling of MSCs does not affect their basic properties, such as the proliferation and the phenotype, and can overcome the variability between donors of their biological activities [16, 17, 21, 31]. In addition, the batch-to-batch variability of pooled MSCs is limited compared with batches produced with MSCs from a single donor, providing an opportunity to standardize medicinal products [22]. Despite several advantages, this strategy may be of limited benefits when the pool consists of randomly selected donors, particularly if several or all of the donors have low biological activity [21]. Thus, prior selection of donors could improve the biological properties of pooled MSCs.

In this study, we graded the profile of donors based on the immunoregulatory properties of the UC-MSCs, in order to select donors whose cells were to be included in pools. Our aim was to prepare pools including UC-MSCs isolated from at least one donor with a high immunomodulatory potential. The selection of a potency assay to evaluate the immunomodulatory potential of MSCs is a critical step. Ideally, this assay should reflect the mechanism of action of MSCs in the targeted

pathology and be related to clinical efficacy. However, it is currently difficult to clearly identify a relevant assay associated with efficacy due to a complete lack of knowledge of the mechanisms of action of MSCs in vivo. Thus, to assess immunomodulatory functions of MSCs, we followed the recommendations of the ISCT, which specifies the use of a standardised immune assay as well as expression surface markers and IDO after in vitro priming with pro-inflammatory cytokines [23]. We have therefore developed and validated such assays that meet the regulatory requirements and can be used as potency assay to qualify clinical batches of MSC-based ATMP. Among these different assays, we decided to use a MLR assay to grade MSC donors because a MLR is considered to mimic an in vivo response like the one found with e.g. GvHD, which is one of the most important indications for MSC therapy. In our MLR assay, MSCs are co-cultured with a pool of 10 PBMC donors to reflect allogeneic activation and to reduce the variability of PBMC donors, thus standardising the assay [24]. Donors were graded as low if the suppression of T-cell proliferation was below the median, as medium if above the median, and as high if the results were in the last quartile. Consequently, five donors were graded as low, three as median, and two as high.

To assess the benefits of pooled UC-MSCs compared with single donors, we pooled cells isolated from one donor of each profile in an equal proportion, based on ratios reported in the literature [16, 32]. First, our results showed that pooled UC-MSCs displayed a similar morphology and phenotype to cells from individual donors. IDO expression was significantly different between the three donor profiles, with the highest expression by UC-MSCs^{high} after treatment with IFNγ and IFNγ + TNFα, despite a similar IFNγ-receptor expression. Indeed, our results demonstrated that the expression of IFNγ-receptor is donor-independent and is not associated with the priming of MSCs by IFNγ as previously shown [33]. Interestingly, pooling UC-MSCs (UC-MSCs^{pool}) increased the lowest expression of IDO (UC-MSCs^{low}), while the expression of the IFNγ receptor by UC-MSCs^{pool} remained unchanged. In addition, IDO expression by UC-MSCs^{pool} was similar to the theoretical mean, highlighting that pooling the cells resulted in homogenization. These results were confirmed by the MLR assay which showed persistence of donor-dependent variability. In the basal state (ratio 1:10), the suppression of T-cell proliferation by UC-MSCs^{high} was higher than UC-MSCs^{medium} ($p > 0.05$) and UC-MSCs^{low} ($p = 0.0278$) whereas UC-MSCs^{pool} tends to increase T-cells suppression compared to UC-MSCs^{low} (Fig. 6A). In the presence of IFNγ (ratio 1:10), T-cells suppression by UC-MSCs^{pool} was significantly higher than UC-MSCs^{low}, UC-MSCs^{medium} as well as the theoretical mean of single donors. Similar results were found after priming cells with IFNγ + TNFα. This suggests that the high profile MSCs enhance the biological activity of the other donor MSCs. This is supported by our results of pooling 2 donors (high and low) in different ratios (1:1 and 1:2) (Fig. 7), which show that this improvement is maintained even when the amount of MSCs from low donor in the pool is twice that of MSCs from high donor. Recently, Kannan et al. suggested an improved immunosuppressive capacity of pooled batches due to synergistic effects between single donors, although this remains debatable as analysis was performed on expanded cells until P5, with unequal proportions between individual donors [31]. At this stage, we cannot explain the mechanism behind our results showing an enhancement of immunomodulatory functions of the pooled MSCs. Our hypothesis is that the MSCs communicate with each other through their capacity for cell–cell and paracrine interactions, allowing the high profile MSCs to confer better activity to the low profile MSCs.

In our study, we showed that UC-MSCs with greater expression of IDO were able to largely inhibit T-cell expansion. Other mediators such as prostaglandin E2 (PGE2), TGF-β, TSG-6 and NO have also been described, underlining the role of the paracrine effect of MSCs to regulate immune cells [34, 35]. Despite the growing interest in paracrine mechanisms, contact-mediated immunomodulatory effects are critical to guarantee the full efficacy of MSCs immunosuppressive functions [26]. Ren et al., showed that MSCs failed to suppress T-cell expansion when they were separated by a permeable membrane, highlighting the importance of contact-dependent mechanisms [27]. The suppression of monocyte differentiation into dendritic cells, the switch of macrophages into an anti-inflammatory phenotype, the differentiation of $CD4^+$ T cells into T regulatory cells and the inhibition of Th17 cells are mediated by cell-to-cell interactions via cell-surface molecules [35–38]. We therefore decided to analyze the expression of immune and adhesion surface markers involved in cell-to-cell contact. First, our results showed a high heterogeneity in surface markers expression depending on the donor. CD200 was high in UC-MSCs^{high}, ALCAM/CD166, CD146 and VCAM-1/CD106 in UC-MSCs^{medium}, whereas PDL-1 presented a similar expression in UC-MSCs^{high} and UC-MSCs^{low} (Fig. 3). Interestingly, despite these variations, pooling UC-MSCs resulted in an increased expression of all these molecules. ALCAM/CD166, CD146 and CD200 were increased in UC-MSCs^{pool} in the basal state and after priming, whereas VCAM-1/CD106 and PDL-1 needed a pro-inflammatory

stimulation even after pooling MSCs. ICAM-1/CD54 was significantly higher in UC-MSCs^{high} at basal state only and was enhanced by IFNγ or IFNγ + TNFα to reach around 100% in all donors and in the pool. Finally, CD44 was homogeneously expressed regardless of the donor and culture conditions. These results suggest that the immunomodulatory functions of UC-MSCs could be increased by pooling cells without pro-inflammatory priming. The immunoregulatory mechanism of pooled cells appears to involve cell–cell interactions through the induction of the expression of adhesion molecules ALCAM/CD166, CD146 and CD200. CD200 expressed on the surface of MSCs has been described to bind to its receptor CD200R expressed on macrophages, leading to their switch into anti-inflammatory phenotype [39]. In our study, the expression of VCAM-1/CD106 and PDL-1 was higher in pooled cells after inflammatory priming, although not in all single donors. ICAM-1/CD54 and VCAM-1/CD106 play a key role in the contact-mediated immunoregulation by MSCs [40], and PD-L1 is involved in the inhibition of T and B-cell activation in a cell-contact manner [41].

Taken together, our results show that pooling of UC-MSCs not only reduces donor-dependent heterogeneity, but also that the inclusion of UC-MSCs^{high} in pools enhances the lowest immunoregulatory properties. These results were confirmed even when the amount of UC-MSCs^{low} was twice that of UC-MSCs^{high}. To our knowledge, this is the first study to demonstrate such results. Therefore, we proposed a method of grading donor based on their immunomodulatory potential then pooling UC-MSCs from multiple donors with at least one high donor in low quantity. Although our strategy may be difficult and time consuming to implement in a clinical application context, it offers some advantages: (i) to preserve as much as possible the stock of precious high donors (only 20% of the donors in our study), (ii) to use these high donors to induce the biological activity of low donors, and (iii) to obtain large batch sizes with high biological activity, without the need to expand high donors at a high passage to obtain therapeutic doses. Indeed, the extensive ex vivo expansion over several passages may introduce the risk of genetic instability and chromosomal abnormalities as well as an alteration of the biological properties of the MSCs [42].

Our study has several limitations. First, because we used clinical-grade cells from MCS, we were only able to include 10 donors in our study, which may limit the generalizability of our results. Although the analyses of single-donors *versus* pooled donors were repeated several times to improve the reliability of the results, it would be necessary to validate our strategy with other donors. Second, we used the donor UC180 as a high donor for assessment of the pool 3D ratio 1:1:1. As this donor exhibits some particular characteristics of collection, the representativeness of this selection may be questionable. However, we also selected another high profile donor (UC185) to perform the experiments in Fig. 7. Although the characteristics of donor UC185 do not represent the collection characteristics of donor UC180, the immunosuppression results of both donors are similar. Third, although UC-MSCs are known to have a favourable safety profile [43], it is indeed possible that pooling MSCs from different donors may increase the risk, in particular their immunogenicity. In our study, we showed that the induction of HLA-DR expression by pro-inflammatory treatment is donor-dependent. Pooling normalized HLA-DR expression without increasing the expression of HLA-DR or the co-stimulatory molecules (CD40, CD86) compared with UC-MSCs^{high}, demonstrating the possibility of using a pooled allogeneic product without altering the tolerogenic profile of UC-MSCs. A non-clinical study performed in rats and rabbits showed the absence of acute or chronic toxicity, tumorigenicity, and teratogenicity following administration of human bone marrow-MSCs pooled from three healthy donors in an equal proportion [17]. Safety data from clinical trials appear also reassuring (19, 20). However, it remains important to evaluate this aspect when developing a pool strategy. Finally, our study lacks in vivo experiments to validate both the choice of an MLR assay for donor grading and the conclusions regarding the consistency and optimization of the immunomodulatory functions of MSCs highlighted by our in vitro experiments. Few studies have evaluated pooled MSCs in in vivo models [32, 44]. Although their pool strategies differ from ours, as the MSCs pools are composed of random donors and not pre-selected donors, this supports the relevance of the approach.

Conclusion

In this study we propose a method to classify donors based on the inhibitory properties of UC-MSCs on T-cell proliferation (graded as high, medium, and low potential), and then prepare pools of UC-MSCs from multiple donors, including at least one donor classified as high potential. Our results demonstrate that this strategy reduces the donor-to-donor variability and improves the immunomodulatory functions of low potential donors, without increasing immunogenicity of the cell product. Thus, our strategy circumvents the issue of the biological heterogeneity of UC-MSCs and opens up new perspectives to produce large scale standardized UC-MSC-based medicinal products, with an improvement in their immunomodulatory properties for the treatment of severe and/or refractory immune or

inflammatory diseases. Our perspective is to confirm this first in vitro proof of concept in an in vivo model.

Abbreviations

ATMP	Advanced therapy medicinal product
AT-MSCs	Adipose tissue-derived MSCs
BM	Bone barrow
BM-MSCs	Bone marrow-derived MSCs
CFU-F	Colony-forming-unit-fibroblast
CTV	CellTrace™ Violet
DMSO	Dimethylsulfoxide
DPBS	Dulbecco's phosphate buffered saline
DT	Doubling time
EMA	European Medicines Agency
GM-CSF	Granulocyte macrophage colony-stimulating factor
GMP	Good manufacturing practices
GMP-ATMP	Good manufacturing practices specific to advanced therapy medicinal products
GvHD	Graft versus host disease
IDO	Indoleamine 2,3-dioxygenase
IFNγ	Interferon-gamma
IL	Interleukin
ISCT	International Society for Cell & Gene Therapy
MA	Marketing authorization
MCS	Master cell stock
MLR	Mixed lymphocyte reaction
MSCs	Mesenchymal stromal cells
MEM-α	Minimum essential medium alpha
PL	Platelet lysate
NT	Non-treated
P	Passage
PBMC	Peripheral blood mononuclear cells
PD	Population doubling
RPMI	Roswell Park Memorial Institute
T-Ly	T lymphocyte
TNFα	Tumor necrosis factor-alpha
UC	Umbilical cord
UC-MSCs	Umbilical cord-derived mesenchymal stromal cells

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-025-04361-y.

Additional file 1. Antibodies used for UC-MSCs staining in the basal state and after pro-inflammatory priming.

Additional file 2. The number of isolated UC-MSCs at P0 depending on the weight (g) of UCs, the gender of babies, and the delivery method.

Additional file 3. Flow cytometry strategy for IDO assessment. A. Gating strategy for the percentage of UC-MSCs expressing IDO. Cells were stained with anti-CD90 antibodies (extracellular staining), then fixed and permeabilized to allow intracellular staining with anti-IDO antibodies. The gating strategy is shown with debris exclusion, selection of UC-MSCs (CD90pos) among the cells and gating of IDOpos expression (%) among the UC-MSCs. B Representative images of IDO expression among UC-MSCs in the basal state (NT) and after pro-inflammatory priming with IFNγ for UC-MSCslow, UC-MSChigh and UC-MSCspool2D.

Additional file 4. Flow cytometry strategy for suppression of T-cell expansion by UC-MSCs. A Gating strategy for identification of proliferative T cells. Cells were stained with anti-CD45, anti-CD3 antibodies and a viability dye (7-AAD). The gating strategy is shown with exclusion of debris and doublets (FSC and SSC), then selection of live cells (7-AADneg), leukocytes (CD45pos), total T cells among leukocytes (CD3pos) and proliferative T cells (CTVpos). For CTV gating, the strategy is based on the point at which the first cell division took place, visible by fluorescence dilution. B Representative images of the proliferation of T cells when a bank of PBMC from 10 donors is cultured without (control) or with different donors of UC-MSCs (UC-MSCslow, UC-MSChigh and UC-MSCspool2D) after pro-inflammatory priming with IFNγ at a ratio of UC-MSC/PBMC 1/30, for 7 days. Gating of proliferative T cells is based on CD3posCTVneg expression.

Additional file 5. IDO expression (%) after priming with IFNγ+TNFα and suppression of T-cells proliferation in the basal state depending on A the age of mother, B the delivery term, C the weight of UCs, D the weight of babies and E the gender of babies.

Additional file 6. UC-MSCs immunophenotype in the basal state. Expression of mesenchymal (CD90, CD73, CD29, CD105), hematopoietic (CD14, CD45) and endothelial (CD31) markers by A UC-MSChigh, B UC-MSCsmedium, C UC-MSCslow and D UC-MSCspool.

Additional file 7. Detailed flow cytometry data.

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Author contributions
M.M conceived, planned and performed the experiments, analyzed the data, wrote the manuscript and supervised the work. CMP planned and performed the experiments, analyzed the data and revised the manuscript. RER, ALP, AS, VF performed the experiments and analyzed the data. JL analyzed the data and revised the manuscript. AC designed and supervised the research, analyzed the data and revised the manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this article and its supplementary files.

Declarations

Ethics approval and consent to participate
For UC samples: (1) Title of the approved project: « autorisation d'activité de conservation et de préparation d'éléments du corps humain en vue de leur cession pour un usage scientifique». (2) Name of the institutional approval committee or unit: Direction générale de la recherche et de l'innovation, Ministère de l'enseignement supérieur et de la recherché. (3) Approval number: AC-2022-5325. (4) Date of approval: 19 December 2022. All mothers have provided written informed consent for the use of samples. For blood samples (PBMC): (1) Title of the approved project: « convention cadre de cession de produits issus du sang ou de ses composants à but non thérapeutique». (2) Name of the institutional approval committee or unit: Etablissement Français du Sang/Inserm. (3) Approval number: CCPSL 2024-2027-001. (4) Date of approval: 2 January 2024. All healthy donors have provided written informed consent for the use of samples.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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