Supplemental Information – Jorgensen et al.

Online supplementary text

Harvesting cell process

ASC were manufactured according to GMP rules using aseptic procedures and disposable sterile single-use supplies for all product contact steps. The cell culture was performed according to cell engineering unit quality system. The whole process was conducted in accordance with written procedures and each step was recorded on batch records. All manipulations involving the initial preparation of cells, cell culture, preparation, and cell packaging were performed in cleanrooms of appropriate class of air cleanliness. All manufacturing staffs were trained in use of the process pertinent SOP including the line clearance and disinfection procedures. The batch production records for each lot required documentation and confirmation signatures that the procedures have been followed.

Adipose tissue was harvested from the subcutaneous abdominal adipose tissue in the operating room under local anaesthesia. The aspiration procedure used standard procedures and the standard equipment routinely used in an operating room. After applying the tumescence solution percutaneously into the abdominal fat depot, two micro-incisions of 3 mm were made. Afterwards a Coleman's cannula armed with a syringe was inserted into the fat depot and 10 to 12 syringes of fat were harvested. More than 60g of sub-cutaneous adipose tissue were removed by aspiration using 10 ml EC marked syringes capped in sterile conditions. Skin incisions were sealed with steristripes to ensure appropriate wound healing. An elasto-compressive bandage was mounted to avoid hematoma. The duration of this procedure was approximately 40 min. At the end of the aspiration procedure, operating room nurse placed an appropriate label on the outside of the syringe. The tissue sample, in its primary packaging, was placed in a secondary container (and then placed in an isothermal container labelled according to requirements laid down in Directive 2004/23/EC). The container was shipped from the clinical site to the manufacturing site using suitable mode of transportation that ensured delivery of the package to the manufacturing site within 24 hours. The transport was done at 5±3°C, with temperature traceability by a certified transporter. The harvested tissue was kept within the syringes until processing.

Cell preparation, expansion of ASCs

ASCs were prepared on the single site of EFS-MP (Toulouse, France), using subcutaneous adipose tissue from abdominal fat after liposuction. Adipose tissue was transported to the EFS-MP at 4°C within 4 hours of harvesting. Briefly the stromal vascular fraction (SVF) was obtained by means of

collagenase digestion. Aliquots of 10 g of adipose tissue were mixed with 34 mL of the collagenase solution (NB6; Coger, Paris, France) and incubated at 37°C for 45 min. Enzymatic digestion was stopped by the addition of complete culture medium (CCM) containing minimum essential medium (MacoPharma, Tourcoing, France), human platelet growth factor enriched plasma, 10 mg/mL ciprofloxacin and 1 U/mL heparin. After homogenization, the digested suspension was passed through sterile 100-mm filters. The cells were centrifuged at room temperature for 10 min at 600g. The supernatant was discarded and the SVF was resuspended in 20 mL of CCM. An aliquot of the SVF was removed for the quality controls: cell count, viability, phenotyping (CD34, CD45 and CD14) and sterility.

The cells from the SVF were then seeded in a 1270-cm^2 CellStack culture chamber (MacoPharma) at a density of 4.10^3 cells/cm^2 in CCM, with the use of seeding kit (MacoPharma), at 37°C in an atmosphere saturated with moisture and 5% CO_2. After an initial 24-h incubation, the non-adherent cells were removed. The adherent cells were washed once with Dulbecco' s phosphate-buffered saline (PBS), and CCM medium was added for 7 days. The medium was completely replaced at day 4 and day 6 of culture with the use of medium exchange kits (MacoPharma). . At day 8 (primary culture, P0), the cells were harvested with the use of a detachment kit (MacoPharma) according to the following protocol: after aspiration of the medium and washing with Dulbecco' s PBS, 50 mL of irradiated trypsin solution was added for 5 min at room temperature. After the inhibition of trypsin activity by the addition of CCM, the cells were collected in a transfer bag (MacoPharma). An aliquot of the cell suspension was aseptically removed for cell count, viability, phenotyping (CD34, CD45 and CD14), measures of hTERT messenger RNA (mRNA) contents by qRT-PCR and assessment of microbial testing.

The cells were seeded in a 1270-cm^2 Cell-Stack culture chambers at a density of 2×10^3 cells/cm^2 and incubated for 6 days. The CCM was completely replaced at day 11 and day 13. At day 11, an aliquot of culture medium was aseptically removed for mycoplasma and endotoxin testing. At day 14, the cells were harvested according to the same procedure as described above. The cell suspension was placed in a transfer bag (MacoPharma) and washed with Dulbecco's PBS. The ASCs were then resuspended in a solution containing 3.6% human albumin provided by Laboratoire français du Fractionnement et des Biotechnologies (Courtaboeuf, France) and a poly-ionic solution containing glucose. An aliquot of the ASC suspension was aseptically removed for cell count, and its quality was evaluated as described above.

Flow cytometry analyses were performed as described below. Briefly, ASCs (2×10^5 cells) were stained with saturating amounts of monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) and their respective isotype controls for 30 min in the dark at 4° C in PBS/0.5% human albumin and 0.1% sodium azide. After washing, the labelled cells were analyzed by flow

cytometry (EPICS XL-MCL flow cytometer; Beckman-Coulter, Nyon, Switzerland). FITC anti-CD14, FITC anti-CD45, PE anti-CD34, PE anti-CD73, PE anti-CD90, PE anti-CD105 and immunoglobulin (Ig) G1 PE and FITC were from BD Pharmingen (Le Pont de Claix, France).

Release criteria

Release criteria were defined as negative for microbial testing on SVF, intermediate product (P0) and final product (P1), negative for mycoplasma testing on adipose tissue and culture medium at day 11, endotoxin testing negative on culture medium at day 11, absence of hTERT detection by qRT-PCR on intermediate product (P0). Finally, on active substance, cellular viability had to be > 90%. The percentage of positive cells for haematopoietic markers (CD45 and CD14) had to be lower than 2% and had to be higher than 90% for CD90 and CD73 and more than 80% for CD105 mesenchymal stem markers. The percentage of positive cells for CD34 marker had to be less than 10%.

Karyotypes analyses have been performed, on final product, for 15 productions. Due to the time required for performing the karyotype, results have been obtained after the release. Karyotypes analyses revealed no clonal abnormalities.

Results for release criteria obtained for the 3 cohorts are presented in tables bellow:

Table 1. Results of quality controls obtained for the 1st cohort – cell dose: 2.10^6 cells

Quality controls	Subject n°1 1.01	Subject n°2 1.02	Subject n°3 1.03	Subject n°4 1.04	Subject n°5 1.05	Subject n°7 2.01
Controls on starting material (Day 0)						
Microbial testing*	Negative					
Mycoplasma testing*	Negative					
Controls on intermediate product (Day 8)						
Microbial testing*	Negative					
hTERT (qRT-PCR)*	Negative					
Controls on culture medium (Day 11)						
Mycoplasma testing*	Negative					
Endotoxin testing*	Negative					
Controls on final product (day 14)						
Cell count* > 5.10^6 cells	$43,4.10^6$ cells	$23,8.10^6$ cells	$31,8.10^6$ cells	$38,4.10^6$ cells	$12,3.10^6$ cells	$28,5.10^6$
Viability* ≥ 90%	99,7%	95.6%	95.0%	94.5%	97.1%	90.8%
CD73* ≥ 90%	99,9%	100.0%	99.0%	99.9%	100.0%	100.0%
CD90* ≥ 90%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
CD105* ≥ 80%	99,9%	100.0%	100.0%	100.0%	100.0%	100.0%
CD45* < 2%	0,3%	0.1%	0.1%	0.03%	0.1%	0.15%
CD14* < 2%	0,5%	0.5%	0.9%	0.5%	1.2%	1.2%
CD34* < 10%	2,7%	1.5%	1.6%	1.9%	7.2%	0.9%
Additional controls obtain after the release						
Microbial testing	Negative					
Mycoplasma testing	Negative					
Endotoxin testing	Negative					
hTERT gene expression (qRT-PCR)	Negative					

* Batch release controls

Table 2. Results of quality controls obtained for the 2nd cohort – cell dose: 10.10^6 cells

Quality controls	Subject n°6 1.06	Subject n°8 2.02	Subject n°9 1.07	Subject n°10 1.08	Subject n°11 2.03	Subject n°12 2.05
Controls on starting material (Day 0)						
Microbial testing*	Negative					
Mycoplasma testing*	Negative					
Controls on intermediate product (Day 8)						
Microbial testing*	Negative					
hTERT (qRT-PCR)*	Negative					
Controls on culture medium (Day 11)						
Mycoplasma testing*	Negative					
Endotoxin testing*	Negative					
Controls on final product (day 14)						
Cell count* > 13.10^6 cells	$153,3.10^6$	$148,0.10^6$	$130,4.10^6$	$146,6.10^6$	$45,2.10^6$	$77,6.10^6$
Viability* ≥ 90%	90.0%	100.0%	93.2%	92.8%	94.9%	90.0%
CD73* ≥ 90%	100.0%	99.6%	99.6%	99.4%	99.6%	99.8%
CD90* ≥ 90%	100.0%	99.5%	99.7%	99.5%	99.6%	100.0%
CD105* ≥ 80%	100.0%	99.6%	99.7%	99.7%	99.6%	99.9%
CD45* < 2%	0.01%	0.1%	0.04%	0.3%	0.2%	0.1%
CD14* < 2%	0.6%	0.5%	0.3%	0.9%	0.4%	0.7%
CD34* < 10%	0.2%	0.3%	0.03%	0.2%	5.3%	1.1%
Additional controls obtain after the release						
Microbial testing	Negative					
Mycoplasma testing	Negative					
Endotoxin testing	Negative					
hTERT gene expression (qRT-PCR)	Negative					

* Batch release controls

Table 3. Results of quality controls obtained for the 3rd cohort – cell dose: 50.10^6 cells

Quality controls	Subject n°13 2.06	Subject n°14 1.09	Subject n°15 1.10	Subject n°16 1.11	Subject n°17 2.07	Subject n°18 2.08
Controls on starting material (Day 0)						
Microbial testing*	Negative					
Mycoplasma testing*	Negative					
Controls on intermediate product (Day 8)						
Microbial testing*	Negative					
hTERT (qRT-PCR)*	Negative					
Controls on culture medium (Day 11)						
Mycoplasma testing*	Negative					
Endotoxin testing*	Negative					
Controls on final product (day 14)						
Cell count* > 53.10^6 cells	$174,0.10^6$	$213,9.10^6$	$153,9.10^6$	$86,5.10^6$	$104,5.10^6$	$93,8.10^6$
Viability* ≥ 90%	94.0%	95.1%	98.0%	98.9%	99.6%	98.8%
CD73* ≥ 90%	99.7%	100.0%	99.9%	99.8%	100.0%	99.8%
CD90* ≥ 90%	99.8%	100.0%	99.9%	99.8%	99.8%	99.1%
CD105* ≥ 80%	99.4%	99.9%	99.9%	99.9%	99.9%	99.8%
CD45* < 2%	0.2%	0.5%	0.15%	0.1%	0.1%	0.3%
CD14* < 2%	0.5%	0.1%	0.65%	0.4%	0.2%	1.6%
CD34* < 10%	0.2%	0.2%	3,1%	9.7%	4%	9.8%
Additional controls obtain after the release						
Microbial testing	Negative					
Mycoplasma testing	Negative					
Endotoxin testing	Negative					
hTERT gene expression (qRT-PCR)	Negative					

* Batch release controls