Table 1 Batch release of BMMSCs

Quality assay	Method	Release criteria
MSC phenotype	Flow cytometry (validated)	≥95% : CD44, CD49e, CD13, CD90, CD73, CD29, CD105 ≤2%: CD14, CD19, CD34, CD45 FYI: HLA-ABC, HLA-DR
Viability	NucleoCounter automatic cell counting system (propidium iodide-based) (validated)	≥90% before freezing
Osteogenic differentiation	*in vitro* diff. assay	+ (FYI)
Adipogenic differentiation	*in vitro* diff. assay	+ (FYI)
Sterility testing	BacT/ALERT 3D aerobe and anaerobe growth (validated)	No growth
Endotoxin levels	LAL assay (validated)	Less than 5 IU/kg patient weight
Cell morphology	Phase-contrast microscopy	Normal
Karyotype	G-band analysis (validated)	46, XX/XY
Population doubling number	Cell yield starting from CFU-F number	≤30

Question 3

The quality of the bone marrow aspirate is analysed with the following methods:

(1) a differential blood cell count is performed with a Sysmex XE-2100 haematology analyzer
(2) sterility testing: aerobic and anaerobic bacterial growth with the BacT/Alert 3D system
(3) stromal cell content using the CFU-F assay

Question 4

Every MSC batch will be tested as follows (Table 1):

Question 5a

Defined yes. Critical assays have been validated (MSC phenotype characterization, viability, sterility testing). Ranges have been defined using results from process development and literature.

Question 5b

The batch will not be released for clinical use. Differentiation potential results are for information only, but will be routinely performed.

Questions 6a and 6b

An *in vitro* immunosuppression assay is performed, but the results are not release criteria (only for information). An in-house developed flow cytometry-based assay measuring inhibition of activated PB-MNCs is used.

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S. Fleury-Cappellesso & L. Sensebé

Question 1

Our centre is located at the French National Blood Services (Etablissement Français du Sang: EFS) in Toulouse. Autologous and allogeneic haematopoietic stem cells (HSC), from peripheral blood (PBSC) or bone marrow (BM), are routinely prepared as type II products.

Since 2006, EFS is involved in clinical trials using mesenchymal stromal cells: (i) for regenerative medicine using autologous bone marrow mesenchymal stem cells (BM-MSC) or adipose tissue-derived stromal cells (ACS) to treat, respectively, cardiac insufficiency and critical limb ischemia; (ii) for treating GHVD using allogeneic BM MSC. These productions could be defined as type I products according to EU regulation 1394/2007/EC.

Question 2

Whether type I or type II products, the donor is always evaluated for potential risks related to the collection procedure by a licensed physician. An informed consent from the donor shall be obtained prior to the collection. Donation, procurement and testing are performed in compliance with French Public Health Code. Donors are evaluated for risks factors for disease transmission by medical history, physical examination and laboratory testing. Within 30 days prior to collection, donors are tested for infection disease agents mentioned in article R.1211-14, R.1211-15 and R.1211-16 of the Public Health Code:

(1) Human immunodeficiency virus, type 1 and type 2 (HIV-1 and HIV-2)
(2) Hepatitis B virus
(3) Hepatitis C virus
(4) Syphilis
(5) Human T cell lymphotrophic virus I
(6) Cytomegalovirus

Depending on donor's history, others tests could be performed as toxoplasma, EBV, West Nile Virus and *Trypanosoma cruzi* (Chagas' disease). All testing procedures are performed in accredited laboratories authorized by regulatory authorities. Identity of the donor is checked by a physician prior to the collection and includes the name, date of birth, age, gender and medical history.

Type I products. Donors are carefully selected according to inclusion and exclusion criteria defined in the clinical study protocol. These criteria should be approved by an ethic committee and French regulation authority before the clinical trial began. For allogeneic uses of BM-MSC and ASC, HLA A, B, C and DR typing is done and could be used.

Type II products. Donors selection and evaluation are performed according to JACIE. For collection of HSC by apheresis, an evaluation of venous statement, a possible need for central venous access and the mobilization therapy is evaluated within 2 weeks prior to the collection. For bone marrow collection, an evaluation of potential risks of general anaesthesia is performed within 30 days.

In allogeneic context, the donor eligibility is determined by a physician after medical history, exam and testing before the recipient's high dose therapy is initiated and before the donor begins mobilization regimen. Allogeneic donor eligibility is determined regarding HLA compatibility with the recipient, serologic status and the potential risk of the collection. HLA typing is performed by a laboratory accredited by EFI and includes HLA-A, B, C and DR type.

Allogeneic donors are also tested for red cell compatibility with the recipient.

Question 3

At the arrival, cell engineering unit verifies the integrity of the packaging, aspect of the product, duration and temperature of the transport, identity of the donor and coherence with accompanying documents.

Type I products. The safety of the clinical trial using BM-MSC & ASC is directly linked to specific controls implemented during the cell processing and depend on starting tissue material [1].

For ASC production, fat tissue weight, cell counting, viability, phenotype analysis and sterility control are carried out [2]. For BM-MSC, bone marrow volume, cell counting, viability, clonogenic potency (CFU-F) and sterility control are performed. For recipient safety, sterility control must be negative.

Type II products. Evaluation of cellular therapy products is based on a total nucleated cell count, viability, CD34+ cells enumeration [3], CFU-GM assay and sterility control.

Question 4

Quality assays and release criteria have to be suitable and compatible with a rapid release of the graft. For this purpose, in some cases, quality control results of previous steps of the processing must be taken into account to release the final product.

Type I products. Quality controls and release criteria have been validated and defined in the clinical study protocol. Final controls for ACS and BM-MSC production include cell counting, viability, phenotype analysis and bacteriological control.

In France, the genetic stability of cultured MSC is checked by karyotyping 30 metaphases. The karyotype is only used as release control when cultured BM-MSC & ASC are cryopreserved and used after thawing. Moreover, MSC being not prone to transformation and karyotype not being completely relevant, we are testing other potential markers as hTERT expression and Q-PCR for different gene products (e.g. p53, p21 & c-myc) [4].

Type II products. Haematopoietic stem cells are released only in view of a nominative prescription. Autologous grafts are validated in the light of quality controls before freezing and include CD34 cell count, viability and bacteriological test. Beside these tests, ABO incompatible allogeneic grafts are evaluated for red blood cell volume.

Questions 5a and 5b

Sterility control of the last stage of the process is known in posteriori because of the time of culture (12 days according to French regulation recommendations). Therefore, sterility control is evaluated at a previous stage, if applicable, and must be negative.

Type I products. Phenotypic criteria for release MSC has been validated on more than 80% of mesenchymal markers expression (for ASC: CD90, CD73 and CD13; for BM-MSC: CD90 andCD73) and the lack of haematopoietic cells (CD45 and CD14 < 5%) [5].

In our centre, MSC are produced for phase I clinical trials. Viability threshold should be more than 70% and more than 75% for, respectively, ASC and BM-MSC production. The cell number should be in accordance with the predefined dose (e.g. 100×10^6 for ASC in limb ischaemia and 80×10^6 BM-MSC in cardiac ischaemia).

Type II products. After conditioning regimen, the minimum dose to achieve acceptable engraftment is 2×10^6 CD34/kg. In case of insufficient dose of CD34+, the final product is released but may induce a delay in haematopoietic recovery. The final decision is always subject to the physician approval for use regarding the urgent medical

need and the absence of therapeutic alternative. In the presence of ABO-incompatibility, separation using density gradient (BM graft) or dilution (PBSC graft), residual red blood cell volume is decreased under 15 ml.

Questions 6a and 6b

Potency assays are not performed prior to release. During the validation phase of processes developed for production of BM-MSC and ASC, two types of potency assays are implemented:

(1) For immunosuppressive activity: using immunological platform developed by INSERM unit U917 for two programs of 7th FP of EC (CASCADE n°223236 and REBORNE n°241879), the mechanisms and effects of MSC (BM MSC & ASC) on effectors of innate and adaptative immunity are systematically tested.
(2) For uses in regenerative medicine, the following assays are used: (i) in bone repair, potential osteoblastic differentiation using our previously reported differentiating method [6], (ii) for vascular repair purposes, dosage of some released cytokines and growth factor (e.g. VEGF).

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H. Bönig

Question 1

Type I products (all ATMPs).

(1) Autologous bone marrow mononuclear cells for cardiovascular regeneration.
(2) Mesenchymal stroma cells (third party, mixed from several unselected donors).
(3) Cytokine-induced killer cells (CIK) (allogeneic stem cell donor derived).

Type II products (non-ATMP cellular therapeutics).

(1) Haematopoietic stem and progenitor cells from marrow (also ery-depleted, volume-reduced, washed), mobilized peripheral blood (also volume-reduced, washed) and cord blood (ery-depleted and volume-reduced) for haematopoietic reconstitution, autologous and allogeneic, also cryopreserved – 'standard' cellular therapeutics, not addressed here.
(2) Matched-donor lymphocytes (DLI), also cryopreserved – 'standard' cellular therapeutics, not addressed here.
(3) Haematopoietic stem and progenitor cells from mobilized peripheral blood, autologous and allogeneic, immunomagnetically manipulated (CD34+ selected, CD3/CD19 depleted), also cryopreserved (unmatched-related or unmatched-unrelated).
(4) Antigen-specific matched-donor or unmatched-donor lymphocytes, immunomagnetically manipulated (Adenovirus, Cytomegalovirus), also cryopreserved.
(5) Femoral head, allogeneic, heat-inactivated, cryopreserved.

Question 2

Type I products.

(1) Autologous bone marrow mononuclear cells for cardiovascular regeneration: Marrow is drawn by treating physician; treating physician does donor assessment with regard to donor safety and indication for cellular therapy, as well as written informed consent. The treating physician fills the collection form and peels unique labels from the collection form, which he attaches to the cellular product as well as to companion blood tubes; he confirms donor identity with his signature on the collection form. IDMs do not play a role in donor eligibility testing for this product.

(2) Mesenchymal stroma cells, third party: Donors are invariably bone marrow donors for 'bone marrow transplantation' from whom we draw an extra aliquot of marrow with their written informed consent and approval by the IRB. Thus, donor eligibility, including IDMs, and donor ID are the same as for marrow donors (acc. to German Stem Cell Guidelines, German Transfusion Law, WMDA standards).

(3) CIK cells: Donors are evaluated as DLI donors, according to national and international guidelines, including eligibility, IDMs, donor ID, etc.

Type II products.

(1) Immunomagnetically manipulated stem cell products: Donor eligibility evaluation is done according to national and international guidelines for stem cell donors, including IDMs, donor ID, etc.

(2) Immunomagnetically manipulated T-cell products: Donor eligibility evaluation is done according to international guidelines for stem cell donors, including IDMs, donor ID, etc. The only additional test is for the frequency of antigen-specific T-cells. Whenever possible (contingent on the presence of at least 1:1000 antigen-specific T-cells), the original stem cell donor is used. If not possible, a reasonably matched (at least haplo-identical) third-party donor is selected from a stem cell donor registry.

(3) Femoral head: Donor eligibility assessment is done by orthopaedic surgeons based solely on donor history. Written informed consent is obtained by the same surgeon. No IDMs are tested prior to donor clearance. The surgeon who removes the femoral head fills the collection form and peels unique labels from the collection form, which he attaches to the product container as well as to companion blood tubes and informed consent forms; he confirms donor ID with his signature on the collection form.

Question 3

Type I products.

(1) BM-MNCs for cardiovascular regeneration: Processing center does serology for Hepatitis A, B, C, HIV, Syphilis on blood. Incoming material is tested for integrity, completeness, CBC (automated haemocytometer), sterility (culture – pending at the time of release).

(2) Mesenchymal stroma cells: Starting material comes with all the pertinent test results for allogeneic BM for stem cell transplantation, including IDMs (because the companion product is cleared and released for transplantation). Incoming BM specimen is additionally tested for CMV genome in the cell pellet as required by the federal overseeing body. Sterility is tested by culture: CBC, volume, total cell number, total MNC number as above.

(3) CIK cells: Volume, CBC, sterility, CD3, CD25, CD56; total cell count is calculated from these data. Day of collection IDMs (serology and NAT).

Type II products.

(1) Immunomagnetically manipulated stem cell products: Starting material is a cleared stem cell product; all the pertinent quality-defining values are available [CBC, viability (7AAD), CD34+, CD3+, Hkt, Volume, IDMs]. No additional quality tests are required.

(2) Immunomagnetically manipulated T-cell products: Starting material is a cleared DLI product – see above. In addition, the frequency of antigen-specific T-cells is again assessed, but values are not available until the end of processing and only serve for quality control purposes (recovery).

(3) Femoral head: Availability of all paperwork and blood for serology and NAT, integrity of the container, proper labelling of all papers, blood tubes and container with unique product number.

Question 4

Type I products.

(1) BM-MNC for cardiovascular regeneration: Outgoing material is tested for CBC (specification: 5–50 × 10E7 TNC, Leuko > 70%), sterility (culture – pending at the time of release). We do not have a potency assay, because we have not identified a non-clinical surrogate potency marker. Not required for release are analyses of *in vitro* migratory potential, CD34, CD14, KDR, CD133, CXCR4 and some other surface markers which are routinely performed, by virtue of which we are hoping, in the future, to identify predictive 'potency markers' for our cell product.

(2) MSCs: Release of MSC products is contingent on the qualification and clearance of the 'master cell bank' from an aliquot of which the clinical product was generated. Clearance of the master cell bank is very complex (including MLR, senescence, freedom from a large panel of viruses and other pathogens, identity through FACS and functional assays) and detailed description of the process exceeds the limitations of this article. The aliquots for clinical use are cryopreserved. They are released based on sterility, as well as FACS analysis prior to cryopreservation for viability by dye exclusion (>90%), CD45, CD14, HLA-DR (all <10%) and CD90, CD73, CD105 (all >90%) and freedom from endotoxin.
(3) CIK cells: Release requires testing for CD3, CD25 and CD56, sterility of a sample drawn 2 days prior to the harvest at the time of release (culture results pending), and a >10-fold increase of the frequency of CD3+CD56+ cells. An aliquot containing a number of potentially allo-reactive T-cells (CD3+CD56−) as defined by the treating physician is released non-irradiated, the remainder is released after irradiation with 3000 cGy.

Type II products.

(1) Immunomagnetically manipulated stem cell products: Measurement of total cellularity, CD34+ cells (CD34-enriched products: >90%, otherwise 'declared') and CD3+ cells (declared), viability (>70%). In CD3/CD19 depleted cell products, additionally residual B-cells and NK-cells are measured. The number of allowable T-cells is defined by the clinician (in writing). Potentially, less than the entire product is released to the patient. The remainder can be cryopreserved for later use. Sterility is tested (culture), but the results will not be available upon release.
(2) Immunomagnetically manipulated T-cell products: Measurement of total cellularity, CD3/4/8+ cells, and % antigen-specific T-cells (by IFN-release after antigen-stimulation or by streptamer technology, depending on the clinical trial; release criterion: >50% purity). The total number of allowable potentially allo-reactive (not antigen-specific) T-cells is defined by the clinician (in writing). Potentially, less than the entire product is released to the patient (dosing based on allo-reactive T-cell contents), the remainder can be cryopreserved for later use. Sterility is tested (culture), but results will not be available upon release.
(3) Femoral head: IDMs negative for Hepatitis A, B, C, HIV, Syphilis by serology, Hepatitis B, C and HIV by NAT, supernatant sterile, all documents available.

Question 5a

Type I and type II products. Yes – see above.

Question 5b

For all products: Per specification, only 90% of products must meet release criteria. When they do not, for all DIRECTED products, clearance and release is possible if the treating physician declares the vital indication in writing. This specifically also applies to autologous products positive for any type of infectious agents, including HIV and Hepatitis, as well as to non-sterile products. Non-directed products (femoral head, MSC) that do not meet release criteria are discarded or may be used for laboratory research.

Question 6a

Type I products.

(1) BM-MNCs for cardiovascular regeneration: No, we have not been able to identify a surrogate marker of potency.
(2) MSCs: Only during validation of the master cell bank, suppression of a mixed lymphocyte reaction is tested and is requisite for clearance of the master cell bank.
(3) CIK cells: We consider the emergence of CD3+CD56+ cells a potency assay. Direct assays are not performed prior to release.

Type II products.

(1) Immunomagnetically manipulated stem cell products: The number of CD34+ cells/kgBW of the recipient is used as potency assays.
(2) Immunomagnetically manipulated T-cell products: detection of IFN-secreting or antigen-binding cells, respectively, is considered potency assays of antigen-specific T-cell products. The accuracy of this assumption, except for some evidence of a dose–response relationship *in vitro* and in a xenograft model, has not been directly assessed.
(3) Femoral head: No, we do not know of such an assay.

Question 6b

Type I products.

(1) BM-MNCs for cardiovascular regeneration: After evidence of its potency in mice, a clinical trial demonstrated the clinical potency of products generated according to our method – notably for the cohort, not for every individual. Markers that can predict potency of a given product have not been identified.

(2) MSCs: Suppression of MLR – see above. Markers that can predict potency of a given product have not been identified.
(3) CIK cells: Suppression of tumour and leukaemia cells in a xenotransplant assay. Markers that can predict potency of a given product have not been identified.

Type II products.

(1) Immunomagnetically manipulated stem cell products: Clinical trials support the use of CD34+ cell dose as a potency assay.
(2) Immunomagnetically manipulated T-cell products: It is not clear whether there is a dose–effect correlation for antigen-specific T-cells, since they proliferate *in vivo* in response to antigen contact. It is reasonable to assume, however, that a larger allo-reactive T-cell dose bears the risk of GvHD.
(3) Femoral head: Sufficient evidence abounds about the clinical potency of heat-inactivated allogeneic unmatched bone grafting using the Telos-method. No additional evidence was sought, nor was any requested by the regulators.

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Question 1

The Center of Transfusion Medicine, Cellular Therapy and Cryobiology (CTMC) includes (i) a public cord blood bank named 'Milano Cord Blood Bank', (ii) a laboratory in charge of processing haemopoietic progenitor cells from bone marrow (HPC-Marrow), HPC from mobilized peripheral blood (HPC-Apheresis) and allogeneic Lymphocyte-Apheresis units, (iii) a GMP laboratory for the preparation of advanced therapy medicinal products (ATMP) in compliance with EU regulation 1394/2007 and (iv) a flow cytometry laboratory performing cell immunophenotyping.

During February 1993 – September 2011, the Milano Cord Blood Bank, which was accredited by the Foundation for the Accreditation of Cellular Therapy (FACT) in 2004, has received and cryopreserved 27 562 and 9465 cord blood units (HPC-CB) from 37 delivery suites, respectively. From the cryopreserved inventory, 467 units (4·9%) have been distributed for unrelated HPC transplantation to 146 transplant centres worldwide. In addition, the bank has stored 461 directed HPC-CB units from families with a relative affected by a condition with an indication to family-related CB transplant. Seventeen (3·7%) of the latter units have been transplanted. On 18 August 2011, the Milano Cord Blood Bank has qualified to participate under the US National Marrow Donor Program (NMDP) Investigational New Drug (IND) application: A Centralized Cord Blood Registry to Facilitate Allogeneic, Unrelated Donor Umbilical Cord Blood Transplantation, effective 20 October 2011. The Milano Cord Blood bank does not perform commercial autologous CB storage. HPC-CB units donated to the Milano Cord Blood Bank are processed with a volume reduction procedure using a Compomat G4 device (Fresenius) [1].

In addition to managing the public HPC-CB allogeneic inventory, the staff of the Milano Cord Blood Bank performs the characterization, cryopreservation and thawing of HPC-Marrow, HPC-Apheresis and Lymphocytes-Apheresis units collected for clinical use in the hospital HPC transplant program, which was accredited in 2010 by the Joint Accreditation Committee-ISCT (Europe) & EBMT (JACIE). During 2010, a total of 64, 33 and 91 HPC-Marrow or HPC-Apheresis units have been cryopreserved, minimally manipulated and thawed, respectively.

The GMP laboratory incharge of ATMP preparation – named 'Franco Calori Cell Factory' – develops experimental products and clinical trials of cellular therapy in cooperation with clinicians of different specialties including haematology, cardiology, hepatology, neurology, orthopaedics, nephrology and neonatology. Since the onset of activities in 2000, the Franco Calori Cell Factory, which was accredited by the Italian Drug Agency (Agenzia Italiana del Farmaco, AIFA) in 2007, has prepared 50 units of ATMP, which have been distributed to five clinical centres for use in five experimental protocols. In addition, approximately twice as many ATMP have been prepared for process validation purposes.

In summary, we prepare the following type I products (i.e. those with substantial manipulations or not intended to be used for the same essential function in the recipient as in the donor): (i) expanded CD34+ cells from HPC-CB purified with the CliniMACS device (Miltenyi, Bergisch Gladbach, Germany) [2]; (ii) CD133+ cells from HPC-Marrow and HPC-Apheresis, purified with the CliniMACS device; (iii) autologous and allogeneic mesenchymal stem cells from adipose tissue (MSC-AT), bone marrow (MSC-Marrow) and CB (MSC-CB), purified with adherence procedures and *ex-vivo* expanded with different protocols.

Moreover, we prepare the following type II products (non-substantial manipulations): (i) HPC-CB for allogeneic transplant; (ii) autologous and allogeneic HPC-Marrow; (iii) autologous and allogeneic HPC-Apheresis; (iv) allogeneic Lymphocytes-Apheresis units.

Table 1 Cord blood testing

Test	Time	Sample
Collected nucleated cell count	Banking	Whole blood withdrawn from collection bag
Cryopreserved nucleated cell count	Banking	Buffy coat after volume reduction
Nucleated cell count	Before unit release	Segment attached to the frozen bag
Nucleated red cell count	Banking	Blood film from buffy coat.
ABO and Rh blood type	Banking	Red cell fraction after volume reduction
Genomic HLA-A,B low resolution and DRB1 high resolution typing	Banking	Segment from the frozen bag detached before freezing
Genomic HLA-A,B high resolution and DRB1 high resolution typing if not performed before (old units). Other HLA loci at requested level of resolution	Upon request when the unit is selected for a patient	DNA extracted from segment attached to the frozen bag
Confirmatory HLA-A,B,DRB1 typing	Before unit release	As above
Maternal HLA-A,B,DRB1 typing	Before unit release	DNA extracted from repository of samples, collected at 6 months check or, if absent, at delivery (EDTA sample)
CD34 + cell count	Banking	Buffy coat after volume reduction
Haemoglobinopathy screening	Banking	Whole blood from collection bag
Cell viability determined if transport temperature is out of range	Banking	Whole blood from buffy-coat bag
Cell viability	Before unit release	Segment attached to frozen bag
Clonogenic potential evaluation: CFU-GM, CFU-GEMM, BFU-E, CFC	Banking	Buffy coat after volume reduction
Clonogenic potential evaluation: CFU-GM, CFU-GEMM, BFU-E, CFC	Before unit release	Segment attached to frozen bag
Microbial culture for aerobic and anaerobic bacteria and fungi	Banking	Unit sample at end of processing prior to cryopreservation, after cryopreservative addition
HBsAg, anti-HCV, anti-HIV 1-2, HCV/HIV1 RNA - HBV DNA NAT and anti-Treponema P. test	Banking	Maternal serum collected at delivery
Anti-HBc (total and IgM) total and IgM, ALT, anti-CMV IgG and IgM and anti-Toxoplasma IgG and IgM test, anti-HTLV I-II	Unit selection for a patient	Maternal serum collected at delivery
HBsAg, anti-HCV, anti-HIV 1-2, and anti-Treponema P. test	Six months check after delivery	Maternal serum sample collected 6 months after delivery
ALT, anti-CMV IgG and IgM and anti-Toxoplasma IgG and IgM test, anti-HTLV I-II	Before unit release	As above
HBsAg, anti-HIV 1-2, anti-HCV, anti-HTLV I-II test	Before unit release	Unit plasma
CMV-DNA, Toxo-DNA test if anti-CMV IgM or anti-Toxoplasma IgM is positive	Before unit release	Segment attached to frozen bag

Question 2

For HPC-CB units collected to enter the Milano Cord Blood Bank inventory - our primary banked product - health donor screening is performed at the delivery suites with a standard donor questionnaire compliant with the national laws, European norms and regulations, and Netcord/FACT standards. Donor selection criteria are focused on risk factors for infectious and genetic transmissible conditions.

Health donor screening for autologous and allogeneic donors of HPC-Marrow, HPC-Apheresis and Lymphocytes-Apheresis units collected in our institution is done in the haematology unit with a standard questionnaire compliant with national laws, Italian Bone Marrow Donor Registry, and JACIE standards. Additional criteria may apply to units received from other institutions, according to local procedures and regulations.

All mothers of HPC-CB donors are screened at banking for anti-HCV, anti-HIV antibodies, HBsAg and Syphilis with standard blood banking serology procedures. Moreover, nucleic acid testing (NAT) for HCV/HIV RNA and HBV DNA is performed at banking. Mothers with positive HCV, HIV, HBV markers are not allowed to donate cord blood for solidaristic allogeneic purposes. Their serology markers negativity is checked 6 months after delivery [3], as prescribed by the current Italian law. Additional testing is performed at unit release, as shown in Table 1, which summarizes HPC-CB testing at the different stages of processing and release and the type of sample used for testing.

HPC-Marrow, HPC-Apheresis and Lymphocytes-Apheresis units screening includes the following: anti-HCV, anti-HIV, anti-HTLV I/II antibodies (the latter is not done for autologous use), HBsAg, anti HBc antibodies and Syphilis. Moreover, nucleic acid testing (NAT) for HCV/HIV RNA and HBV DNA is performed. Sterility for aerobic and anaerobic bacteria and fungi is determined with the Bactec method.

Selection of autologous donors for experimental ATMP protocols is performed by the clinicians incharge of the

patient treatment according to specific inclusion and exclusion criteria of each experimental protocol.

Storage of autologous cryopreserved ATMP from donors with positive HCV markers, who currently participate in an experimental clinical protocol for the treatment of end-stage liver disease, is done in the vapour phase of dedicated liquid nitrogen tanks.

Other infectious markers can be determined upon request of the clinical centre using the product depending on patient characteristics, treatment protocols and national regulations of the requesting centre.

Donor identity checking for HPC-CB, in addition to standard practices of direct donor questioning and unit and samples barcode labelling, is done through comparison of unit HLA typing at banking with confirmatory typing at time of product release. The latter is done on a segment integrally attached to and stored with the bag.

Additional procedures to reduce the risk of unit misidentification include the use of separate areas for unit processing and storage and for administrative activities, the rule to manage one kind of product at any one time and the use of double checks by two operators at critical steps, for example, before detaching two connected bags and when the product is transferred into a new container. All procedures for sample collection requiring the entry of the bag are performed with aseptic techniques under a sterile flow hood.

Question 3

General rules for the characterization of quality of the starting material include the following: (i) inspection and evaluation of the integrity of the package containing the material; (ii) coherence and completeness of material and donor information reported on the material container, the associated samples and the accompanying forms; (iii) evaluation of the compliance between the conditions of material transport to the CTMC including time and temperature with specific limits for each material.

In addition, assays specific for the different materials include the following.

Type I products. (i) HPC-CB to obtain expanded CD34+ cells; (ii) HPC-Marrow and HPC-Apheresis to obtain CD133+ cells; (iii) AT, HPC-Marrow and HPC-CB for the production of autologous and allogeneic MSC: cell counting (internal method); flow cytometry (internal method); viability with Propidium Iodide (P.I.) by flow cytometry; sterility testing (Eu. Ph. 2.6.27). In addition, for expanded MSC, we perform HLA typing (class I), karyotype and telomerase activity.

Type II products. (i) for HPC-CB see Question 2; (ii) autologous HPC-Marrow, HPC-Apheresis: volume, total nucleated cells (TNC), haematocrit, CD34+ cell count and viability, haemopoietic colonies (CFU-GM and CFC) in methylcellulose; in addition, CD3+, CD4+, CD8+, CD19+, NK cell count are determined in allogeneic collections; (iii) Lymphocytes-Apheresis: volume, total nucleated cells (TNC), haematocrit, CD3+, CD4+, CD8+, CD19+, NK cell count and viability.

Question 4

Before releasing any product, we perform a comprehensive check that all the steps during the procedure from collection to release have been performed correctly and are properly documented. In addition, quality control testing of the final cellular therapy product prior to release includes a number of assays as follows.

Type I products. (i) CD133+ cells from HPC-Marrow and from HPC-Apheresis: cell counting (internal method); flow cytometry (internal method); viability with P.I. by flow cytometry; sterility testing (Eu. Ph. 2.6.27) and endotoxins (Eu. Ph. 2.6.14); (ii) expanded CD34+ cells from HPC-CB as in (i) plus mycoplasma (Eu. Ph. 2.6.7); c) MSC: as in (ii), plus karyotype and telomerase activity. Moreover, different potency assays are performed in different protocols (e.g. cytokine secretion and migration assays); in the case of allogeneic use, mixed lymphocyte reaction with recipient's mononuclear cells is performed on multiple MSC lots to screen the best unit for the recipient.

Type II products. (i) HPC-CB see Question 2. Specifically, an integrally attached segment is used to determine nucleated cell count and viability, CFU-GM and CFC. Moreover, we also determine HPC-CB mother's genomic HLA-A,B,DRB1 typing at low resolution to check mendelian inheritance of HLA type; (ii) HPC-Marrow and HPC-Apheresis (vial sample): TNC count and viability (trypan blue), CFU-GM and CFC; Lymphocytes-Apheresis: TNC count and viability.

Question 5a

We have defined the following acceptable ranges/thresholds of the results of release assays.

Type I products.

(i) cell count: for all the products, the range depends on the requested cell dose; (ii) viability (P.I.): ≥80%; (iii) sterility and mycoplasma: for all the products, the results must be 'no growth'; (iv) endotoxins: ≤2·5 IU/ml; (v) flow cytometry: for expanded CD34+ cells from HPC-CB, the absolute value of CD34+ cells depends on the requested cell dose; for CD133+ cells, purity must be ≥80%; for MSC-AT, MSC-Marrow, MSC-CB, the percentage of CD90+/105+/73+/45- cells must be ≥90%; (vi) karyotype: 46;XX or 46;XY (normal karyotype).

Type II products. HPC-CB: see Question 2. Although we do not define a minimum TNC/kg of patient's body weight as

a formal release criteria, we do not recommend to transplant HPC-CB units with a dose below $2{\cdot}5 \times 10^7$ TNC/kg. Acceptable criteria for release of HPC-CB, HPC-Marrow and HPC-Apheresis are any CFC growth and cell viability ≥50%. In the very uncommon cases of 'no colony growth', we inform the clinician and repeat the assay. The final decision to use or not to use the unit is taken by the clinician, who balances the risks and benefits for the recipient. Release criteria for Lymphocytes-Apheresis units are cell viability ≥50%.

Question 5b

Type I products. A specific policy is defined in each clinical trial.

Type II products. See 5a.

Question 6a

Potency assays at release are as follows.

Type I products. For ATMPs, apart from the assays performed using flow cytometry, we do not perform additional potency assay at release.

Type II products. The potency assay for HPC's is the CFC count; see 5a. For Lymphocytes-Apheresis, apart from the immunophenotype, we do not perform any potency assay at release.

Question 6b

See Question 6a.

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M. Takanashi

The following answers refer only to the Japanese Red Cross Kanto-Koshinetsu Cord Blood Bank, in the Japanese Red Cross Kanto-Koshinetsu Block Blood Centre (formerly in the Japanese Red Cross Tokyo Blood Centre).

Question 1

We deal with cord blood, which is a type II product.

Question 2

Type II product. Questionnaires are filled out by the expectant mothers who volunteer, on their and their family's health history. One of the questionnaires is similar to that given to blood donors, while the other is unique for cord blood donations and inquires about the family history of inheritable diseases. Inheritable diseases in close relatives which can be transferred to the recipient by stem cell transplantation are considered, depending on the mode of the inheritance pattern, such as X-linked recessive, etc. When there have been multiple familial cancers, mother's malignancy or autoimmune disease, the cord blood is not collected. If the delivery and the newborn's condition are without problems, then the cord blood is collected.

Question 3

Type II product. At the start, we require a collected volume of more than 60 ml of cord blood. We start processing the cord blood when the total nucleated cell count (TNC) with an automated counter (XE-2100; Sysmex, Hyogo, Japan) is $11{\cdot}4 \times 10^8$ or more, or if the TNC is between 10 and $11{\cdot}4 \times 10^8$ and the CD34 positive cell count is $4{\cdot}4 \times 10^6$ or more with 7AAD/CD45/CD34 staining using Stem-Kit (stemCXP v2.0/FC500; Beckman Coulter, CA, USA) [1].

The mother's serum is sent to the test section for infectious marker screening, which includes HBs, anti-HBc, anti-HCV, anti-HIV-1/2, anti-HTLV-1, parvovirus B19, syphilis and anti-CMV using chemiluminescence enzyme immuno assay (CLEIA; CL4800, Fujirebio, Tokyo, Japan). Using cord blood plasma separated after it is mixed with 20v/v % HES, the same serological screening tests with CLEIA are performed as well as nucleic amplification tests for HBV, HCV and HIV-1/2. When the mother's anti-CMV IgG/IgM is positive, we perform an anti-CMV IgM EIA (Denka Seiken, Tokyo, Japan) using the cord blood plasma, which will be replaced by a CMV DNA detection test using a sample of the cord blood.

Question 4

Type II product.
(1) We have final product regulations for placing the cord blood into our open search system:

Sterility tests are performed with (i) the Oxoid Signal Blood Culture System (Oxoid, Cambridge, UK) using 0·5 ml of the final product mixed with DMSO, and with BacT/ALERT, (ii) BPA and (iii) BPN culture bottles (bioMérieux, Lyon, France) using 10 ml for each from the removed RBC fraction.

The TNC using an automated counter, the CD34 positive cell count using 7AAD/CD45/CD34 staining and the CFU-GM count (MethoCult H4034; Stemcell Technologies, BC, Canada) are posted for each cord blood unit.

For identification, the ABO and Rh typing, forward blood typing only, and HLA allele typing (Luminex 200; Luminex, Austin, TX, USA) are performed.

(2) Before releasing the unit, we carry out the following tests using a test sample:

(i) TNC with an automated counter, (ii) total cell viability with ethidium bromide and acridine orange (EB/AO) fluorescence counting, (iii) CD34+ cell counting with 7AAD/CD34/CD45 staining, and (iv) stem cell culture assay using MethoCult H4034.

The viability of the total nucleated cells should be 70% or more. The recovery rate of CD34 positive cells compared with before freezing should be 60% or more.

Question 5a

Type II product. The cell count recovery should be 80% or more. The viability of the total nucleated cells should be 70% or more. The recovery rate of CD34 positive cells compared with before freezing should be 60% or more. The recovery rate of the total CFU compared with the total CFU before freezing should be 50% or more, though the CFU assay result is used only for quality control, and not for the release decision.

The recovery tests for 10 057 units had mean ± SD of 99·8 ± 7·9% for the TNC, 82·2 ± 6·0% for the viability, 79·8 ± 19·5% for the CD34 positive cells and 76·0 ± 23·0% for the CFU.

Question 5b

Type II product. When the viability or the recovery rate of CD34 or CFU does not meet the criteria, we do the test again with the segment sample attached to the cryobag.

In the cases when the TNC recovery and cell viability is satisfactory but one of the other criteria is not met, and the prefreezing test result looks to have a possible overcounting, we suggest to use the cord blood unit. Otherwise, we search for other units and suggest to the transplantation centre to change the donor cord blood unit.

Question 6a

Type II product. (As above for Question 5a), the viability of the TNC should be 70% or more. The recovery rate of CD34 positive cells compared with before freezing should be 60% or more. The recovery rate of the total CFU compared with the total CFU before freezing should be 50% or more, though the CFU assay result is used only for quality control, and not for the release decision.

Question 6b

Type II product. Not applicable.

Reference

1 Keeney M, Chin-Yee I, Weir K, *et al.*: Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE guidelines. International Society of Hematotherapy and Graft Engineering. *Cytometry* 1998; 34:61–70

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S. M. Watt, E. B. Austin & M. Guttridge

Question 1

National Health Service Blood and Transplant (NHSBT) is a national special health authority for England and North Wales in the UK, taking responsibility for the supply of blood, tissues, blood-related stem cells and their products and organs for the National Health Service. The information provided will cover the cellular therapies facilitated by the NHSBT Stem Cell and Immunotherapy (SCI) Department which encompasses (i) the NHS Cord Blood Bank with its six cord blood collection sites at Watford General, Barnet, Northwick Park, Luton and Dunstable, St George's, and University College NHS Hospitals which are in or close to London and has a collection hub at Colindale in London and a processing and banking facility in Bristol, and (ii) seven SCI laboratories which receive, process, manufacture, manipulate, bank and distribute haemopoietic stem cells and related cellular products for therapeutic use for approximately 50% of haemopoietic stem cell transplants in the UK. The latter laboratories are based in Birmingham, Bristol, Leeds, Liverpool (previously Manchester), Oxford, Sheffield and Southampton and are an integral part of NHSBT. Each provides a regional hub for the routine provision of such cellular products for transplantation. For example, Birmingham SCI serves five transplant units and

a population of ca. 2 million patients in the West Midlands. As well as the routine transplant services, the SCI laboratories have established themselves as a link between R&D programmes within NHSBT and academic centres of excellence within Universities and NHS teaching hospitals for the provision of cell therapeutics. The products listed are a snapshot of recent or ongoing clinical applications involving specific NHSBT SCI laboratories or departments.

Type I products include:

(1) chimeric T cells for treating haemopoietic and other cancers.
(2) dendritic cell therapies for treating cancers [1].

Type II products include:

(1) harvests of unrelated and related sibling allogeneic umbilical cord blood units (HPC, Cord Blood) are collected, tested, as necessary processed for volume reduction and removal of plasma and red cells and cryopreserved at <−150°C prior to selection for transplantation [2].
(2) related and unrelated allogeneic bone marrow harvests (HPC, Marrow) are processed, red cell depleted and cryopreserved at <−150°C and/or reinfused into patients [3, 4].
(3) autologous or related and unrelated allogeneic peripheral blood harvests mobilized with G-CSF with or without Plerixafor (HPC, Apheresis) are processed and cryopreserved at <−150°C and/or reinfused into patients [3, 4].
(4) allogeneic mobilized peripheral blood harvests (HPC, Apheresis) may also be T/B (CD3/CD19) cell depleted [4].
(5) allogeneic or autologous mobilized peripheral blood (HPC, Apheresis) harvests may also be CD34/CD133 selected for the treatment of haematological, cardiovascular or liver diseases [4].
(6) allogeneic donor therapeutic T cells (T-CT) and CMV-specific T cells may be selected using magnetic bead technology and reinfused into patients to treat or preempt CMV infections [5].
(7) CD25 depletion of peripheral blood cells for treating patients with cancers [6].

Question 2

Background to compliance with regulatory requirements within NHSBT SCI Departments. The regulatory requirements for cellular therapies have increased exponentially in the past few years in England, where the regulation of Type I and II products is split between different competent authorities. Routine stem cell and immunotherapy processing and minimal manipulation of cell therapy products (Type II products) as carried out in NHSBT SCI Departments are regulated by the Human Tissue Authority (HTA) and, as a legal requirement, each NHSBT SCI laboratory is now licensed by the HTA. Additionally, new scientific progress in cellular and molecular biotechnology has led to the development of advanced therapies (AT), such as gene therapy, somatic cell therapy and tissue engineering. New cellular therapies or advanced therapy medicinal products (ATMPs) offer opportunities for groundbreaking new treatments for many diseases/injuries and depend on more extensive manipulation of cell therapy products, here referred to as Type I products. Under the current EC framework, ATMPs are defined as products which have the properties to treat or prevent a disease or which may be administered to restore, correct or modify physiological functions via a pharmacological, immunological or metabolic action. Their evaluation requires specific expertise beyond that needed for routine NHSBT SCI service provision and conventional medicines, and the legislation is designed to ensure that this expertise is available to generate these products. This has more recently led to the requirement for adherence to regulations for ATMPs via the Medicines and Health Products Regulatory Agency (MHRA) licensing in England. The existence of different competent authorities regulating cell therapy products for human application has led to the development of a UK Stem Cell Toolkit for therapeutic cell manufacture and research (http://www.sc-toolkit.ac.uk/home.cfm).

HTA licensing of cellular products. The NHSBT SCI departments are licensed by the Human Tissue Authority (HTA) as the competent authority in England for handling human cells intended for human application. This is a legal obligation that has been enacted into criminal law in England. The principal HTA guidelines based on EU Directives and legislation and covering the activities of the NHSBT SCI Departments are listed.[1] Under HTA Directions 001/2006, donor selection criteria are based on an analysis of risks related to the specific cell or tissue application and where risks are identified for living donors by, for example, medical and behavioural history review, biological testing and physical examination. Donors are excluded based on a risk assessment where there is evidence of risks for transmissible diseases and where donor travel and exposure and the prevalence of local infectious disease are considered. The HTA provides regulatory alerts and advice on donor selection to licensed establishments handling human cells intended for human application (http://www.hta.gov.uk/licensingandinspections/regulatoryalerts.cfm). One recent example relates to donors visiting West Nile Fever endemic areas where, in relation to this, the HTA endorsed the 'Change Notification by the Joint UKBTS/NIBSC Professional Advisory Committee (JPAC) to the UK Blood

Transfusion and Tissue Transplantation Services Donor Selection Guidelines'.

Regulatory requirements for advanced therapy medicinal products (ATMPs) – Type I products. Where a cell therapy product is substantially manipulated (e.g. by gene therapy, tissue engineering or for somatic cell therapy), it is classed as an advanced therapy medicinal product (ATMP) for human use and categorized as a biological medicinal product in Annex I to Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use and regulations and Directives listed.[2] In addition, where appropriate and taking into account the specificities of ATMPs, additional requirements have been set out as follows:

(1) specific requirements for gene therapy medicinal products;
(2) specific requirements for somatic therapy medicinal products;
(3) specific requirements for tissue engineered products;
(4) specific requirements for advanced therapy medicinal products containing devices.

Legally in England, the MHRA is the competent authority for licensing establishments for the manufacture of investigational ATMPs or Type I cellular therapy products currently produced in NHSBT. It should be noted that ATMPs are regulated by the HTA in terms of donation, procurement and testing of human tissues and cells, while the MHRA regulate the manufacture, storage and distribution of the Type I CT product. While all NHSBT facilities are HTA licensed, the first MHRA licence for ATMPs involving an NHSBT SCI Department was granted in 2011 to the NHSBT Advanced Therapy Unit (ATU) in Liverpool, England.

FACT-Netcord, JACIE and FDA accreditation for Type II CT products. For routine NHSBT SCI minimally manipulated processing of cells for transplantaion (Type II CT products), all NHSBT SCI Departments are voluntarily FACT-Netcord (for unrelated cord blood banking) or JACIE accredited. These require adherence to standards, which must also be compliant with individual country's legal requirements.[3] The World Marrow Donor Association (WMDA) first developed standards specifically for unrelated donors. These WMDA standards can be found on the WMDA website (http://www.worldmarrow.org).[4] Unrelated cord blood donations exported by NHSBT to the USA also fall under the U.S. Department of Health and Human Services Food and Drug Administration (FDA) requirements. In October 2009, Center for Biologics Evaluation and Research produced final guidance for cord blood bank applications for licensure of minimally manipulated, unrelated allogeneic placental/umbilical cord blood, for specified indications entitled 'Minimally Manipulated, Allogeneic Placental/Umbilical Blood Intended for Hematopoietic Reconstitution for Specified Indications' (http://www.fda.gov/cber/guidelines.htm).[5] At this time, there are no public cord blood banks licensed by the FDA, and cord blood donations can only be used for transplantation in the USA through an Investigational New Drug (IND) exemption granted by the FDA to USA transplant centres and the NMDP. The NHS Cord Blood Bank has been approved by the NMDP to export via their IND exemption.

Other guidelines. Other guidelines covering NHSBT SCI Departments are (i) Guidance on the Microbiological Safety of Human Organs, Tissues and Cells used in Transplantation - advisory committee on the microbiology safety of blood and tissues for transplantation (SaBTO - Department of Health, England), (ii) Guidelines for the Blood Transfusion and Tissue Transplantation Services in the UK (Red Book ESD/PTI/001) and its associated supplements containing donor selection guidelines (http://www.transfusionguidelines.org.uk), and (iii) UK Blood Transfusion Services and NIBSC Tissue Donor Selection Guidelines Cord Blood.

Donors Release 08–21 June 2011 [UKBTS and NIBSC TDSG-CB 2003 (1 June 2007) Release 08 (21 June 2011)] produced by the Joint UKBTS/NIBSC Professional Advisory Committee (JPAC) of the UKBTS and NIBSC.

Donor selection criteria. For both Type I and II Products manufactured in NHSBT SCI Departments, the following apply as minimal requirements for donors:

Health history screening.
As detailed in the HTA (Quality and Safety for Human Application) Regulations 2007, HTA Directions, extensively in the UKBTS and NIBSC TDSG-CB 2003 (1 June 2007) Release 08 (21st June 2011) and in the SaBTO guidance, selection criteria for donors are based on an analysis of risks related to the specific cell or tissue application. All cell therapy products are tested for bacterial contamination from the environment during manufacture and storage.

Allogeneic and autologous donors are selected on the basis of their health and medical history and their physical status, with additional information obtained from healthcare specialists where possible. An interview is provided by a trained and qualified healthcare professional with the donor to assess medical, behavioural and travel history and with a donor questionnaire. This is then documented by the NHSBT SCI Department.

Exclusion criteria will differ for autologous and allogeneic donors depending on their health status and treatment needs. For allogeneic living donors (see specified guidelines for full details), exclusion or assessment

criteria include history of disease of unknown aetiology, history of malignant disease, risk of transmission of spongiform encephalopathies and other transmissible diseases, travel history outside the UK, systemic infection which is not controlled at the time of donation, any treatment received before donation, any history of blood, blood components, blood products, tissue or organ receipt, previous or current immunosuppression, plasma dilution, infections such as HIV, acute and chronic HBV, HCV, HTLV I and II or children aged less than 18 months born to mothers with HIV, HBV, HBC or HTLV, a history of chronic, systemic autoimmune disease which could be detrimental to the cells/tissues to be retrieved, exposure to substances that may endanger health, recent history of vaccination with a live attenuated virus, and transplantation with xenografts. Other specific exclusion criteria may also be added depending the cells being donated and could, for example, in some instances include pregnancy, breastfeeding and the potential for transmission of inherited conditions.

Infectious disease testing.
For donors of autologous cells that are stored or cultured, the same minimum biological testing requirements apply as for allogeneic living donors, although positive test results may not prevent storage, processing or re-implantation of the cell therapy product.

The following biological tests are carried out as a minimum requirement for the NHSBT SCI Department in accordance with HTA Directions and Guidance on the Microbiological Safety of Blood, Tissues and Organs (SaBTO) (http://www.dh.gov.uk):

(1) for autologous and allogeneic Type I and II products (except for cord blood) within 30 days prior to donation, HIV 1 and 2 using anti-HIV-1,2 testing, HTLV-I and II antibody testing, Hepatitis B virus (HBV) using HBsAg and anti-HBc testing, Hepatitis C virus (HCV) using anti-HCV antibody testing and Syphilis by serology using CE-marked kits according to HTA Directions;
(2) for both the above products, it is the NHSBT SCI Department's policy that testing is repeated on the day of collection or up to 7 days post-donation and that additionally NAT testing for HIV RNA, HBV DNA and HCV RNA is also performed on this sample;
(3) for cord blood donations, the above biological tests listed in (i) are carried out as a minimum requirement on the donor mother's blood sample on the day of birth or up to 7 days after birth, together with nucleic acid testing (NAT) for HIV and HCV (RNA) and HBV (DNA). Prior to release of a cord blood unit for transplantation, samples stored from the cord blood donation are also tested for the infectious disease markers using both the serological testing described above and NAT (HIV and HCV RNA; HBV and CMV DNA);
(4) additional infectious disease testing may be done depending on donor history or the donated cells or the cell therapy application, including malaria, toxoplasma, CMV, EBV, *Trypanosome cruzi*. For cord blood donations, additional tests that are routinely carried out on the day of birth or within 7 days of birth include CMV IgM and IgG antibody testing, with *Trypanosome cruzi* and malaria tested if indicated by maternal travel history.

Identity checking.
For each living donor, donation records contain as a minimum the age, sex, donor identification including first name, family name and date of birth. Where a mother and child are involved in the donation, the name and date of birth of the mother and the name (if known) and date of birth of the child are included. For unrelated donors of haemopoietic progenitor cells and related products used in the routine haemopoietic stem cell transplant setting and where these are collected by a bone marrow donor registry, the donor's name is not made available to the NHSBT SCI Departments, but this is replaced by an alternative identifier that is a unique donor number.

In addition, the NHSBT SCI Departments use the ISBT-128 bar coding system with unique bar code numbers for each donor and their donations to ensure traceability. Thus, for donations from within the UK, all donations are tracked using ISBT128 bar coded identifier labels, together with at least three forms of donor identity viz. date of birth, name or unique donor identifier, and NHS number.

At the time of procurement, the primary cell container/cryobag contains labels specifying the donation identification or code and the type of cells procured, together with the date and if possible the time of donation. For autologous donations, the label also states 'for autologous use only'.

Question 3
The assays performed to characterize the quality of the initial collection for both Type I and II products overlap considerably as they are primarily focussed on safety of the patient and the potency of the collection to meet the desired therapeutic benefit. As such the following relates to both Type I and II products derived from similar sources but, although general principles apply, specific examples are biased heavily towards HPC Type II products which are our main cell therapy products (see also Questions 4–6).

Identity checking. On receipt of a therapeutic cell donation, the first critical check is to confirm the identity of the donation and the intended recipient. This is done by

checking the bag label and accompanying paperwork against donor and recipient details as presented on the referral form provided by and signed by the transplant physician. Any discrepancies or anomalies are referred back to the transplant centre. As described above, the primary identification used for HPC donations, our main cell therapy products, is a unique ISBT 128 donation number assigned at the time of donation. For autologous and related donors, the donor name, date of birth and hospital number are also used, whereas for unrelated donors, where the donor's name should not be disclosed to the recipient, the donor number assigned by the registry can be used as an alternative to the donor name.

The assay most commonly used to confirm identity of the HPC donation is ABO Rh blood grouping. This test is required for JACIE accreditation. However, the test is limited as an identity check because some blood groups occur at high frequency in all populations, and where autologous donors are heavily transfused with compatible blood of a different group, anomalous results can occur and identity of a donation cannot be confirmed. Additionally, CE-marked blood grouping methods may not be validated or approved by the manufacturers for use with HPC, Apheresis, HPC, Cord Blood or HPC, Marrow products. As a consequence, ABO Rh grouping to confirm identity is performed on peripheral blood samples taken from the donor at the same time as the donation itself.

The short tandem repeat (STR) assay provides a more robust test of identify and is used to confirm identity of unrelated donor HPC, Cord Blood donations held by the NHS Cord Blood Bank. Here, the sample used to determine the HLA data for matching is compared with a line segment taken from the cord blood donation itself. If the two samples have identical STR patterns over the 16 loci tested, identity is confirmed. Alternatively, the HLA type can be repeated on the line segment taken from the bag and identity confirmed if the HLA type is identical to that previously recorded for the donation. This test is not used routinely to confirm identity of other therapeutic collections.

Initial cell quantification and purity. It is important to establish the quantities of the active cell populations in initial cell collections as this provides a baseline for assessing recovery of cells after processing and predicts cell doses likely to be achieved in the final product at release to the clinic. Where initial cell counts are poor, it may be necessary for further collections to be requested. Purity is not usually considered relevant in initial collections but is essential in assessing enriched products prior to release for use in the recipient. Donations are assessed on receipt in the NHSBT SCI laboratory using a sample taken from the tubing line attached to the donation. A validated procedure is required to take a 0·5–1 ml sample to ensure it is representative of the donation and that there is no exposure to the environment or risk of bacterial or fungal contamination. The sample is tested initially using a haematology analyser to determine the WBC count, haematocrit (HCT) and platelet count. Some haematology analysers identify all nucleated cells as white blood cells, and where nucleated Red Blood Cells (nRBC) are present, such as in HPC, Cord Blood and HPC, Marrow donations, this may be problematic. The quantity of active cells expressing specific markers, such as CD45, CD34, CD133, CD3, CD19, CD25 or CD56, is determined by flow cytometry. For HPC donations, a single platform method based on the ISHAGE gating strategy is used where CD34+ HPC are defined by their forward and side scatter characteristics, high CD34 and low CD45 expression. The CD45 cell count should be similar to the WBC result from the haematology analyser although it may be necessary to subtract nRBC that may be present in HPC, Marrow or HPC, Cord Blood donations.

The RBC content is also important where an ABO incompatibility is identified between the recipient and the donor, or where there is the possibility of RBC overload in the recipient. The volume of RBC in the graft is determined from the haematocrit (HCT) which is calculated from the mean cell volume (MCV) and the RBC count by the haematology analyser. The relatively high WBC concentration in HPC, Apheresis collections is sufficient to elevate the MCV and RBC count reported by some haematology analysers causing the RBC content to be significantly overestimated. The recipient ABO antibody titre is also determined for ABO incompatible grafts to assist in assessing the transfusion risk should RBC not be reduced to a safe level. The removal of RBC is significantly affected by the presence of storage artefacts, such as RBC crenation. To reduce this, incompatible HPC, marrow grafts should be shipped and stored refrigerated to preserve RBC structure [3].

Potency and viability. For haemopoietic stem cell transplants, the potency represents the ability of the HPC donation to engraft after transplantation. In most instances, this is directly related to the HPC content and cell viability. For all HPC donations, CD34+ and/or CD133+ cells are determined by single platform flow cytometry using the ISHAGE gating strategy with the results expressed as a dose per kg recipient weight. For a durable engraftment, a viable CD34+ cell dose of $>2 \times 10^6$ per kg recipient weight is recommended for HPC, Apheresis collections. The minimum CD34+ cell dose required to achieve engraftment for HPC, Marrow donations is not known due in part to the difficulties in reliably assessing CD34+ cells in bone marrow. Although CD34+ cells are not useful in assessing potency of HPC, Marrow, counting CD34 bright cells by flow cytometry

before and after processing can be helpful in assessing recovery of CD34+ cells and ensuring potency of the graft is maintained after volume or RBC reduction. Other cell markers, such as CD3 or CD19, are also determined by flow cytometry as and when required to assess risk of GvHD or EBV-induced lymphoproliferative disease in recipients. Recommendations for CD34 content of HPC, Cord Blood are described in [2].

Viability is determined using a vital dye, 7-aminoactinomycin-D (7-AAD), incorporated into the flow cytometry assay. The dye is able to penetrate the membrane of non-viable cells specifically staining the DNA which is then visible in the flow cytometry assay. A fresh HPC donation would be expected to have a CD34+ cell viability exceeding 95%. In our facility, CD34 viability below 95% would be investigated further, and in some cases, potency assessed using a colony forming unit (CFU) assay. The CFU assay measures the ability of the cells to proliferate and differentiate.

Sterility. In our facilities, sterility of donations is assessed using the BacT/Alert system. Ideally, 5% of the HPC collection should be used for sterility testing. Where cell doses are suboptimal, this may be reduced to ensure potency of the graft is preserved. In exceptional circumstances, where cell doses are very poor grafts may be released without sterility testing under a medical concession, depending on patient need. Mycoplasma testing if this is done is as described below.

Question 4

Type I products. A major quality release assay for Type I products is that of apoptosis and viability. This is more often done by flow cytometry with both Annexin V and 7-AAD used in combination, allowing detection of early apoptotic cells. This distinction is particularly important in cultured cells as 7-AAD alone can give a falsely low indication of cell viability being reliant on a loss of cell membrane integrity.

Type I products are often cultured for varying lengths of time and this adds to the risk of bacterial contamination. The presence of pyrogens such as endotoxins from the walls of Gram negative bacteria can result in severe patient reactions. Where possible, a test for pyrogens such as the limulus test is recommended. However, the results may not always be available prior to issue. There are three methods for the limulus test: the gel clot test based on gel formation, the turbimetric test based on development of turbidity after cleavage of an endogenous substrate and the chromogenic technique based on the development of colour after cleavage of a synthetic peptide-chromogen complex. In some cases, a Gram stain can be done to look for the presence of bacteria. However, this type of test should be done with caution as the level of bacteria may be below required detection limits especially if the contamination is at a very early stage.

A second contaminant that may develop in cultures is that of mycoplasma which can be present with no obvious change in the host culture, even when the concentration of mycoplasma exceeds that of the host cells by 10–100-fold. The quickest pharmacopoeia method for detecting mycoplasma contamination is that of co-culturing indicator cells such as Vero cells with supernatant from the test culture. After 3–5 days, the Vero cells are harvested, cultured on cover slips then stained with Hoechst stain and examined under fluorescence microscopy. Quicker and potentially more reliable PCR assays are becoming available but at present are not fully recognized as a pharmacopoeia technique.

Type II products. The general principles when developing quality control and release criteria are centred around potency and safety. The product should maintain the potency to achieve the therapeutic benefit intended and be safe for the recipient to receive. For example, HPCs defined as CD34+, CD133+ cells must be at a dose sufficient to achieve a durable engraftment. Equally, the product must be safe to infuse where risks of disease transmission and side effects, such as GvHD and ABO-mediated transfusion reactions, are minimized. Testing requirements for Type II products are outlined below.

Generic release criteria applied to all Type II HPC cellular products.

In all cases, HPC donations must achieve the following:

(1) Contain sufficient viable active cells as requested by the transplant centre.
(2) Sterile as determined minimally using the BacTAlert or similar system.
(3) Negative for all relevant virology markers and syphilis.
(4) ABO blood group compatible or contain <10 ml of incompatible RBC.

Additional release criteria for CD34 enriched or CD3/CD19 depleted allografts.

(1) The CD3 dose should be reduced sufficiently to minimize the risk of GvHD in the recipient. The transplant centre will often state a CD3 dose on the referral/prescription that is used as part of the release criteria. Where the CD3 dose is below the specified dose additional donor CD3+ T cells may be added to achieve the required dose.
(2) Purity should be >90% CD34+ cells. Contaminating cells in CD34 enriched products are usually B

lymphocytes and monocytes. If purity is below 90%, there is an increased risk of EBV-induced lymphoproliferative disease, and the donation is issued based on clinical need as a medical concession. Where CD3/CD19 cells are depleted, purity is not specified in the release criteria.

(3) The recovery of CD34+ cells after processing should be sufficient to maintain potency of the graft. Where CD34+ cell recovery is poor, the processing records would be reviewed to establish the likely root cause and where the transplant dose is below that requested by the transplant centre re-processing to recover more cells may be attempted.

Additional release criteria for cryopreserved HPC grafts.

Cryopreservation is a critical process where further assessment is restricted by sample availability. Line segments and cryovial samples are prepared and stored for each HPC bag although these are not considered representative of the final clinical product due to the differing thermodynamics of the tubing and cryovials compared with the clinical grade cryostorage bag. As such, poor viability identified in the line segment or cryovials may not necessarily identify poor viability in the HPC product itself, although this is usually inferred. To reflect these limitations, release criteria are based primarily on pre-cryopreservation data and satisfactory processing. JACIE recommends that viability post-cryopreservation is assessed, although for well-established validated procedures it may not be necessary to test every donation. Bags are not necessarily discarded on the basis of these results unless a reason for poor viability can be established. Where the procedure is not well established or extensively validated, every donation is tested prior to release to confirm viability post-cryopreservation. There are procedures established to define actions in the event of a significant warming event during storage. These actions include viability assessment by 7-AAD dye exclusion and CFU assessment of some or all affected donations.

Additional release criteria for cryopreserved HPC, cord blood.

HPC, Cord Blood donations stored by the NHS Cord Blood Bank are rigorously tested prior to release for transplantation. This is required as there is limited opportunity to assess the donor's suitability for use as a cord blood donor. The donation may be stored for many years before it is used for a transplant. To reduce the risk of disease transmission from donor to recipient the donor mother, within 7 days of donating HPC, Cord Blood is required to complete health and lifestyle questionnaires and is tested for markers of blood borne diseases mandated at the time of donation. Samples are stored from the mother and the HPC, Cord Blood donation for future use if testing requirements change while the donation is in storage. To further reduce the risk of disease transmission HPC, cord blood donors are screened for haemoglobinopathies. To reduce risks of a transfusion reaction, the donations are also tested to confirm the ABO Rh blood group. To allow matching with potential transplant recipients, the donations are HLA typed to intermediate resolution for HLA-A, B and C loci and to high resolution for DRB1. In addition, approximately 12 weeks after donating the mother is contacted to confirm the health of the infant donor.

When an HPC, Cord Blood donation is identified for a potential recipient, further tests are required prior to release to the transplant centre as follows:

(1) There is a complete review of all paper records including the processing records and freezing curve for the HPC, Cord Blood donation.
(2) A line segment taken from the bag is thawed and tested to confirm viability by dye exclusion with 7-AAD and CFU assays.
(3) Remaining blood from the line segment is used to confirm identity of the HPC, cord blood donation using STR analysis.
(4) The donor's general practitioner (GP) is contacted to confirm the donors continuing health and suitability for transplantation of their HPC, Cord Blood donation.
(5) The HLA type is confirmed and/or tested to higher resolution as required by the transplant centre.
(6) The HLA type of the donor's mother's is determined to confirm the family relationship with the HPC, Cord Blood donors.
(7) Additional mandatory markers for blood borne diseases not required at the time of donation.
(8) Stored samples from the HPC, Cord Blood donation are tested for all mandatory markers of blood borne diseases.

Question 5a

Type I products. For Type I cell therapy products, the release criteria can be multifold and are dependent on the type of cell therapy. For investigational medicinal products, the release criteria will have been specified in the clinical trials authorization (CTA) submission to the MHRA. The CTA will specify the target dose of cells to be given, and this will increase with each cohort in a dose escalation study. Once the cells have been harvested, they will be tested for viability and expression of relevant antigen. For T cell cultures, a viability of >50% is often set with both Annexin V and 7-AAD dual staining by flow cytometry.

Where cell expansion is involved, measurement of the cell number at an early stage in the cell culture is a good indicator that the cells are healthy and expanding well. For our T cell cultures, we looked at the expansion between days 5 and 8, during which time we would expect the cells to have expanded by at least 2·5-fold. For cell therapies involving genetic modification, it is important to know that at least a certain proportion of the cell population is expressing the transgene. Due to the nature and variability in obtaining high levels of transduction, a low acceptance level is set. For example, in the chimeric receptor trial, the acceptance level was set at more than 20% CD3 positive cells expressing the transgene. Along with transgene expression, it is also important that the therapeutic cells are capable of functioning; thus, for the chimeric receptor T cells, the antigen-specific upregulation of CD25 was measured with a cut off of >50%.

One of the most crucial release criteria, however, is that of sterility. With cultured products, it is not possible to obtain sterility results on the final day of harvest when the cells are administered immediately. In this case, a Gram stain negative result is acceptable with the recipient often given broad spectrum antibiotics. A bacteriology test such as BacT Alert is also undertaken but the result will take a few days to be known. Alongside a negative Gram stain result, the cells must also be negative for mycoplasma testing. In addition to the above, the donor cells will have been tested for markers of transmissible diseases which for allogeneic donors must be negative. Autologous marker positive cells can be returned to the donor although in certain trials marker positivity is often an exclusion criterion for entry into the trial.

Type II products. For Type II non-engineered cells where a selection process has been undertaken, there are different criteria dependent on whether the source material is apheresis derived or from bone marrow. Where the transplant physician has specified a cell dose on the prescription referral documentation, then this would form part of the release criteria. Where the dose is not specified as a general rule, for CD34 selection from an apheresis collection, the aim is for a purity of >90% CD34+ cells with >50% CD34 recovery and a > 4 log reduction in CD3 count. For HPC products undergoing a CD3 depletion, the target is to achieve >4 log reduction. Where a combination of CD3 and CD19 depletions is undertaken, the aim is to reduce CD3 by >3 logs and CD19 by >2 logs as a minimum. For marrow collections, volume reduction using a Cobe Spectra should result in a volume reduction to 20% of the start volume with >50% CD34+ cell recovery. For CD34 selection on a marrow collection, the collection volume is first reduced, then the recovery criteria are the same as that for apheresis collections.

Question 5b

Type I products. For Type I products used for clinical trials, the results are discussed with the Sponsor of the trial for a decision on how to proceed. In the majority of cases, the cells would be rejected as they would be outside the specification agreed within the clinical trials authorization. However, if the clinician incharge of the patient wished to transplant the cells in the best interests of the patient, then the cells would be issued under a concession following a written request. In these circumstances, the patient would then be excluded from the trial.

Type II products. For Type II products, the results would be discussed with the transplant consultant and cells issued under concession by the NHSBT SCI Department if the transplant unit makes the decision to proceed.

Question 6a

Type I products. For Type I assays, it is more often not possible to undertake potency testing of the final product due to the necessity to transplant the cells within a short time frame from culture harvest.

Type II products. Specific potency assays are not performed for HPC transplants or CD34/CD133 selected HPCs as these are well established cell therapies, and the gold standard for a haemopoietic stem cell potency is long-term engraftment after transplantation in patients. Surrogate assays are described below. For other products such as CMV-specific T cells, the purity of such cells is assessed by using CMV-specific tetramers and flow cytometry [5].

Question 6b

Type I products. For type I products, the product validation phase can involve analysis for the presence of activation markers such as CD25 which is upregulated following interaction of antigen with specific receptors on T cells. This indicates that the cells are responsive to the target antigen and thus have the potential to mount a response upon transplant into the patient. This responsiveness can also be tested by the use of cytotoxicity assays in which target cells that express the nominal antigen can be co-cultured with the cell therapy product and cell killing of the target cell determined either by a chromogenic assay or dye exclusion or flow cytometry. Other cell therapy products such as dendritic cells can be tested for their ability to stimulate reactive cell responses in immune cells such as T and B cells. These can be measured by the production of cytokines measured by ELISA or ELISpot assay. Where appropriate, such

analyses as sensecence testing, karyoptye, chromosomal integration, toxicity testing, etc., may be required by the regulatory authorities as part of the CTA for Type I products especially where cells are cultured or genetically modified.

Type II products. Potency is in part based on the number/dose of active cells and their viability as measured by dye exclusion using 7-AAD. CMV-specific T cell analyses are described (see [5] and references therein). For HPC collections, CFU assays are still a useful measure of the ability of progenitor cells to proliferate and differentiate and hence provide an additional viability measurement of progenitor cells within the graft. In our establishment, a durable engraftment in the patient should be achieved with defined minimum cell doses. For autologous HPC, Apheresis collections, the minimum dose required to achieve a durable engraftment is 1×10^6 per kg CD34+ cells. For allogeneic grafts, a minimum dose around 2×10^6 per kg is usually considered sufficient although higher doses may be advised if an HLA mismatched donor is used. There is no minimum CD34+ cell dose required to achieve engraftment for HPC, Marrow donations but as a rule, we collect 15–20 ml of bone marrow per kg recipient weight. Viability of CD34+ cells by dye exclusion should exceed 95% in fresh grafts and 80% in cryopreserved grafts. It is a requirement for JACIE and FACT-Netcord accreditation to monitor engraftment in transplant patients. This information provides the most accurate measure of potency and overall performance of the transplant programme.

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End Notes

Type I products: Engineered cells or tissues and Type II products: Non-engineered cells or tissues.

1. (i) Human Tissue Act 2004 and the Human Tissue Act 2004 (ethical approval, exceptions from licensing and supply of information about transplantation) Regulations 2006, (ii) HTA Directions 003/2010, (iii) consent in accordance with the Human Tissue Act 2004, the Human Tissues (Quality and Safety for Human Application) Regulations 2007 and the HTA Codes of Practice (http://www.hta.gov.uk) including HTA Code of Conduct: Consent and HTA Code of Practice: Donation of organs, tissues and cells, (iv) EU Directive 2004/23/EC of the European Parliament and Council on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells, (v) Directive 2006/17/EC implementing Directive 2004/23/EC of the European Parliament and Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells, (vi) Directive 2006/86/EC implementing Directive 2004/23/EC of the European Parliament and of the Council as regards traceability requirements, notification of serious adverse reactions and events and certain technical requirements for the coding, processing, preservation, storage and distribution of human tissues and cells.
2. (i) Directive 2001/83/EC: rules which are applicable to the various types of combinations of medicinal products and devices; (ii) Directive 2004/23/EC of the European Parliament and of the Council: standards of quality and safety for donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells for the manufacturing and distribution of ATMPs; (iii) All biotechnology medicinal products in the UK are now regulated at European Community level under the flagship of the European Medicines Agency (EMA), as established by Regulation (EC) No 726/2004 of the European Parliament, which has set out a centralized authorization procedure, evaluating the quality, safety and efficacy of each produc; (iv) Advanced therapies in Europe are regulated by Regulation (EC) No 1394/2007 applicable from 30th December 2008, which defines ATMPs and how ATMPs are authorized, supervised, and monitored to ensure quality, safety and efficacy (www.ema.europa.eu; advancedtherapies@ema.europa.eu; http://www.ema.europa.eu/SME/SME application.htm; http://www.ema.europa.eu/htms/human/advanced_therapies/certification.htm). This provides precise legal definitions, as well as the specific technical requirements for quality, preclinical and clinical data needed in order to demonstrate the quality, safety and efficacy of the product, ensures free movement of ATMPs within Europe, facilitates access to the EU market and fosters the competitiveness of European companies, guaranteeing the highest level of health protection for patients; (v) Commission Directive 2009/120/EC amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal

products for human use as regards ATMPs was formally adopted by the Commission on 14th September 2009.

3. Standards developed by FACT (the Foundation for Accreditation of Cell Therapy) in the USA and by JACIE (the Joint Accreditation Committee of ISCT (Europe) and the EBMT) or by Netcord (FACT-Netcord International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release. 4th edition) in Europe contain requirements detailing selection, evaluation and management of donors (http://www.factwebsite.org).
4. FACT-NetCord is also involved with the WMDA and the American Association of Blood Banks in the Alliance for the Harmonisation of Cellular Therapy Accreditation, which aims to harmonize international standards.
5. Use is intended for unrelated transplants for haematological malignancies, certain lysosomal storage and peroxisomal enzyme deficiency disorders, Hurler syndrome (MPS I), Krabbe disease (Globoid Leukodystrophy), X-linked adrenoleukodystropy, primary immunodeficiency diseases, bone marrow failure and thalassaemia.

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L. S. McLaughlin

Question 1

We prepare Type II products only. The starting material is derived from apheresis and/or whole-blood collections.

Question 2

For autologous products, the donor/patient is selected by the clinician/hospital that has contracted with us to perform the collection. A brief physical exam and health history is performed before each collection to determine whether the donor/patient can tolerate the apheresis procedure cardiovascularly and that the haematocrit and platelet count are sufficient to proceed without a transfusion. The donor/patient is also examined for signs of an underlying infection to protect the quality of the collected product. The referring clinician determines whether a particular WBC or TNC is required before each collection procedure. Infectious disease testing may be required by protocol or by state statute. The donor/patient's identity is checked against the patient chart or enrolment form prepared by the referring clinician. A government issued picture ID must be presented. If there is a discrepancy between the name stated on the picture ID (even an initial versus the full middle name), the referring clinician/customer is contacted to confirm the identity of the donor/patient and agree to the collection proceeding.

Allogeneic donors are subjected to the same process for autologous donors, except that they are also tested for infectious diseases. For directed donations, the patient's physician may determine whether the donor will be accepted even with positive viral markers.

If the starting material is derived from a whole-blood collection, the allogeneic donor must meet the same criteria as that for a volunteer blood donor. The unit is also tested for the mandatory viral markers before release to further manufacturing. These tests include: HBsAg, anti-HCV, HCV RNA, anti-HIV-1/2, HIV-1 RNA, anti-HTLV-I/II, WNV RNA and a serologic test for syphilis.

Question 3

Depending upon the material, we take samples for sterility and initial cell count before further manipulation or freezing. Sterility is monitored using the BacT/Alert system (bioMérieux, Inc., Durham, NC, USA) CD 34 content is determined by flow cytometry.

Question 4

BacT Alert readings are used to determine whether sterility has been maintained. CD 34 content (if applicable) is determined by flow cytometry.

Question 5a
Yes.

Question 5b
If the thresholds of release are not met, the product is deemed to be 'non-conforming'. The ordering physician or manufacturing customer is notified, and the decision to distribute or destroy the product is made by that party.

Question 6a
No.

Question 6b
We do not perform any other assays other than those described in Question 4.

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