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Use of umbilical cord blood progenitor cells as an alternative for bone marrow transplantation

The use of progenitor cells from cord blood (CB) as an alternative for bone marrow transplantation is one of the important recent developments in transfusion medicine. It has several advantages, such as the unlimited availability of donors, the ability to store large numbers of units in the frozen state, better results with human leucocyte antigen (HLA)-mismatched donors and the reduced danger of graft-versus-host disease (GvHD). However, there is also the problem of cell dose for adult recipients and the slower haematopoietic reconstitution.

In 1998, when the use of cord blood was still in the early stages of development, we carried out a first International Forum on the subject. Since then, a great deal of experience has been obtained and strict International Standards for cord blood collection, testing, banking, selection and release have been adopted. (International standards for cord blood collection, processing, testing, banking, selection and release. Netcord and the Foundation for the accreditation of haematopoietic cell therapy, 2000).

Although the situation is therefore now quite different from 3 years ago, there are, nevertheless, still many aspects of the subject that have not been settled and about which information from experts would be interesting. We therefore sent the following questions to experts in the field:

Question 1. Do you agree that the most important factor in predicting a positive outcome for a transplant is the number of nucleated cells infused?

Question 2. Do you consider the detection of maternal HLA antigens, others than those encoded by the inherited maternal haplotype, a criterion for rejecting a cord blood unit, and which are your other criteria for excluding a cord blood donation?

Question 3. Do you have a programme to evaluate the mother and the baby some time after the donation in order to detect any development which would necessitate rejecting the cord blood sample?

Question 4. Do you apply *in vitro* expansion of progenitor cells and, if so, what are the indications and which technique is used?

Question 5. What are your criteria for accepting autologous and allogeneic directed donations?

Question 6. Can you confirm that, after a transplantation of unrelated cord blood in children, often with one to four HLA mismatches:

(a) neutrophil and platelet recovery occurs later than after bone marrow transplantation;

(b) early post-transplant mortality is higher;

(c) the occurrence of acute or chronic GvHD is lower, even in the presence of major HLA-class I and/or class II mismatches;

(d) that the long-term outcome is not worse than after bone marrow tranplantation;

(e) that there is no correlation between the number of HLA mismatches and survival;

(f) that there is no difference in leukaemia relapse after cord blood or bone marrow.

Question 7. Have you used cord blood transplantation in adults and, if so, what were the results?

Question 8. Do you think that HLA incompatibility unfavourably influences engraftment?

Question 9. Do you think that HLA incompatibility is related to the severity of GvHD?

Question 10. Do you have any experience with the use of haematopoietic growth factors to improve the speed of engraftment?

There is general agreement that the total nucleated cell dose is the most important predictor of the clinical outcome of a transplantation of cord blood progenitor cells. It is mentioned (see Navarrete) that it has been reported that the content of colony-forming cells is a better predictor, but this needs confirmation. In one centre (see Wagner) a threshold CD34 cell dose has been defined; the breakpoint is $1{\cdot}7 \times 10^5$/kg. Above this dose, the incidence of engraftment and the probability of survival do not markedly change. It is mentioned that, whereas cell dose is the only factor predictive of engraftment, other factors, such as age and severe GvHD, also influence transplantant-related mortality. No significant association was seen between the number of HLA mismatches and graft failure (Laughlin). Samples in which maternal HLA antigens are detected, other than those encoded by the inherited maternal haplotype, are rejected in some centres, because the risk of GvHD may be increased. In Milan (see Lecchi *et al*.), the decision is left to the clinician

because the unit concerned may be the only chance of a transplantation for a particular patient. In three centres (Navarette, McClelland, Wagner), testing for non-inherited maternal antigens is not carried out routinely and, in two others (Beguin and Laughlin), samples containing such antigens are not rejected, the evidence for their clinical importance being insufficiënt. For other exclusion criteria, see the answers.

In all centres but two (Wagner, Laughlin), a programme is in place to evaluate the mother and infant at some time-point after donation; this varies from 8 weeks to 6 months. In one centre such evaluation is only carried out when a unit has actually been selected for transplantation. *In vitro* expansion of progenitor cells is not yet applied routinely, but in one centre (Kröger) one patient has been successfully transplanted with *in vitro*-expanded cells and in another (Lecchi *et al*.) *in vitro* expansion is successful, but not yet clinically applied. At the University of Minnesota (Wagner) a phase I clinical trial to evaluate the toxicity and potential efficacy of *ex vivo*-expanded cells is about to start. It is pointed out (Laughlin) that clinical trials have not demonstrated improved kinetics of engraftment in patients receiving expanded cells and concerns about their use are mentioned.

Autologous or directed allogeneic donations are only acceptable under strict conditions and are dependent on a clinical diagnosis, which makes it probable that either the infant itself or a sibling or other relative will later require a transplantation of progenitor cells. In Belgium, autologous donation is not allowed. Although few new data are given in the answers, all contributers agree that neutrophil and platelet recoveries occur later after cord blood transplantation than after bone marrow transplantation. With regard to GvHD and survival rates, the results differ. Whereas in one study no differences were found, in another early survival rate and the incidence of GvHD were decreased after cord blood transplantation; further analyses are therefore required (see Wagner). In adult recipients, early mortality was higher and the incidence of GvHD, both acute and chronic, was lower (Laughlin). Opinions differ on the issue of whether the number of HLA mismatches is correlated with survival. A clear correlation was found in one centre (Navarrete), and in another (Kröger) the number of patients who received cord blood with three or four HLA mismatches was too small to reach a definite conclusion. Other investigators found no such correlation. Published data indicate that the relapse rates of leukaemia after cord blood and bone marrow transplantation are the same. At the University of Minnesota, no difference in relapse was seen in recipients with acute lymphocytic or acute myelocytic leukaemia (Wagner). An important question is whether in adults cord blood is an acceptable alternative to bone marrow. The data show that the overall survival in adults following cord blood transplantation is ≈ 30%, which is considerably lower than in children. Survival was correlated with the nucleated cell dose and severity of disease. Similar results were obtained by Navarrete, Kröger and Wagner. The general opinion is that, in spite of the lower survival rate, cord blood is an acceptable alternative in adults if no matched allogeneic unrelated bone marrow donor is available. The survival rate may improve if the cell dose is increased by *ex vivo* expansion, double cord blood tranplantation or co-injection of mesenchymal stem cells. Each of these strategies is being tested in a phase I trial at the Univerity of Minnesota (see Wagner). In Cleveland, where the major focus of the transplant programme is the study of HLA-mismatched cord blood transplantation in adults with haematological disorders, the long-term outcome is also ≈ 30% survival, but this is similar to the outcome in patients who are similar in age and disease severity, who received an HLA-matched adult donor graft. Although in one centre (Navarrete) no association between HLA disparity and engraftment was observed in a small number of patients, most of the evidence supports such an association. One contributor feels that there is a threshold effect of HLA matching (see Laughlin). There is, as yet, no evidence that the severity of GvHD after cord blood transplantation is related to HLA incompatibility. It is pointed out (Navarrete) that the effect of HLA mismatches on the outcome of cord blood transplantation has been difficult to evaluate owing to the different techniques and the degree of resolution used in HLA typing in the different centres. In Belgium, all recipients, and in Cleveland, most recipients, receive granulocyte colony-stimulating factor (G-CSF). In another centre (Wagner) all recipients receive Neupogen (5 μg/kg/day) until the absolute neutrophil count exceeds 2500/μl, although the effect of prophylactic growth factor has not been found to be significant.

In conclusion, it seems that cord blood is now an established alternative for bone marrow (certainly in children, but also in adults) when a matched unrelated allogeneic bone marrow donor is not available, although the percentage of survival in adults is, at present, only 30%. However, this survival rate may improve if the cell dose is increased by the measures mentioned above. To find definitive answers to some remaining important questions, such as whether early mortality is increased and the incidence of GvHD decreased after cord blood tranplantation, and the influence of HLA disparity, additional analyses are needed.

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Question 1

Cell dose is a significant factor predictive of haematopoietic recovery and engraftment, risk of treatment-related mortality and survival. This result has been previously reported by our group and others. While cell dose is the only factor predictive of engraftment in an analysis of results at the University of Minnesota, age and development of severe acute graft-versus-host disease (GvHD), as well as cell dose, are associated with the incidence of transplant-related mortality, and human leucocyte antigen (HLA) match and development of severe acute GvHD, as well as cell dose, are associated with the probability of survival.

Importantly, we have defined a critical threshold CD34 cell dose below which engraftment and survival are exceedingly poor. For patients at the University of Minnesota, it would appear that the break point for CD34 cell dose is $\approx 1{\cdot}7 \times 10^5$/kg. Once this dose is exceeded, the incidence of engraftment and probability of survival do not markedly change (although the rate of neutrophil recovery continues to improve with increases in cell dose). For these reasons, graft selection is principally based on cell dose rather than HLA match within the group of umbilical cord blood (UCB) units mismatched at < 3 HLA A, B, DRB1 antigens.

Question 2

Criteria for rejecting an UCB unit may differ between blood banks and transplant centers. Blood banks have a minimum set of standards that most often include volume, cell dose, genetic and infectious disease history and microbiological testing. Transplant centres, however, probably have additional standards that are patient specific, such as cell dose and degree of HLA mismatch. However, additional standards may be important at specific centres, such as results of additional testing that might include: repeat HLA typing, number of colony-forming units, enzyme levels (in the setting of inborn errors of metabolism), absence of maternal cells, and feedback on the neonatal and early childhood history of the UCB unit donor. At the University of Minnesota, a unit identified as potentially suitable on the basis of cell dose and HLA match could still be rejected on the basis of:

(1) absence of blood bank registration with the US Food and Drug Administration, FAHCT, or other recognized review body; or

(2) inability to obtain an adequate specimen for repeat HLA typing or specific genetic disease testing.

To date, our centre has not required testing for maternal cells.

Question 3

Prospective studies are needed to determine the impact of maternal/infant testing and evaluation at 6 months or 1 year after the infant's birth. As a Transplant Center, the results would be of interest but the specific question does not apply to this Blood and Marrow Transplant Program.

Question 4

Ex vivo expansion culture is currently being explored at the University of Minnesota as one potential strategy for increasing the time to haematopoietic recovery, reducing non-relapse mortality and improving survival. Using a combination of high-dose flt-3L and thrombopoietin and a synthetic medium to mimic the marrow microenviroment, our group is about to start a phase I clinical trial to evaluate the toxicity and potential efficacy of this approach. There are no data to report at present.

Question 5

Currently, autologous UCB units are only accepted by the Cell Processing Laboratory of the University of Minnesota's Blood and Marrow Transplant Program in the setting of specific genetic diseases for which there may be haematopoietic stem cell gene therapy in the near future (e.g. forms of severe combined immune deficiency, Fanconi anaemia). However, thus far, only four such units have been collected between 1995 and 2001.

In contrast, allogeneic UCB is routinely accepted from families in whom a relative exists that might benefit by haematopoietic stem cell transplantation in the future. In most cases referred to the University of Minnesota, another child within the family is currently being treated for leukaemia or genetic disease. This practice is increasing dramatically as a consequence of investigations with preimplantation genetic diagnosis, i.e. to 'create' a genotypically HLA-identical sibling donor.

Question 6

After transplantation of unrelated cord blood in children, often with one to four HLA mismatches:

(a) neutrophil and platelet recovery occur later than after bone marrow transplantation;

(b) early post-transplant mortality is higher;

(c) the occurrence of acute or chronic GvHD is lower, even in the presence of major HLA class I or class II mismatches;

(d) long-term outcome is not worse than after bone marrow transplantation;

(e) there is no correlation between the number of mismatches and long-term survival; and

(f) there is no difference in the leukaemia relapse rate after CB or bone marrow transplantation.

To date, only two retrospective analyses have been performed comparing results between unrelated donor UCB and bone marrow transplants. Barker *et al.* [1] recently reported the results of a matched-pair analysis at the University of Minnesota. Briefly, patients were matched on the basis of age (> 3 years), disease, risk group (malignancy patients) and

preparative regimen. Patients were not 'matched', however, in terms of donor–recipient HLA disparity (i.e. outcomes in recipients of HLA 0–2 antigen-mismatched unrelated donor UCB were compared with outcomes in recipients of HLA-matched unrelated donor marrow). Notably, the majority of UCB recipients received a one-antigen disparate graft. To summarize the results of this study, neutrophil recovery was delayed, and both GvHD and survival rates were similar in recipients of UCB as compared to marrow. In contrast to the report by Rocha *et al.* [2], early mortality was no different between groups. While this institutional study is benefited by homogeneity in patient assessment and treatment, the registry study is benefited by larger numbers.

Clearly, additional analyses are required. The impact of cell dose, HLA disparity, class of disparity, etc., are being explored in a collaborative registry study between the International Bone Marrow Transplant Registry and the New York Blood Center [3].

No formal comparison has been reported evaluating the effect of stem cell source on incidence of relapse. While analysis of the dataset at the University of Minnesota does not reveal any difference in relapse rates between UCB and marrow recipients with acute lymphocytic leukaemia or acute myelocytic leukaemia, no analysis has been performed that matches risk group.

Question 7

As of November 2001, 51 adults had undergone transplantation with UCB. Of 22 recipients of a single UCB unit treated with a fully myeloablative therapy, the 1-year survival was 30% (95% CI: 11–50). As stated above, new strategies are being explored to 'functionally expand' the cell dose, as this has previously been shown to impact survival. Three strategies are currently being explored: *ex vivo* expansion culture; double UCB transplantation; and co-infusion of mesenchymal stem cells with UCB. Each of these strategies is being tested in a phase I trial. Preliminary data have been reported at the American Society of Hematology meetings in 2001. Furthermore, the use of UCB after non-myeloablative therapy is also being explored.

Question 8

Any impact of HLA disparity on engraftment has yet to be demonstrated within the dataset at the University of Minnesota. While the data would support the contention that UCB lymphocytes are less alloreactive (i.e. less graft-versus-host reaction), it may be that with larger patient numbers an effect of HLA disparity will be demonstrated in the host-versus-graft direction.

Question 9

Any impact of HLA disparity on GvHD also remains to be defined. The dataset at the University of Minnesota shows no relationship between degree of HLA disparity and severity of GvHD. However, it should be noted that the vast majority of donor–recipient pairs at this institution were mismatched at one or two HLA antigens. Too few pairs were matched or mismatched at three antigens. Therefore, it is possible that with greater patient numbers in general, and greater heterogeneity in donor–recipient HLA matching, an effect of HLA matching on GvHD will be observed.

Question 10

Based on the early experience with UCB transplantation in the sibling donor/recipient pair setting, the use of haematopoietic growth factor did not appear to be beneficial. For this reason, haematopoietic growth factor was not used 'prophylactically' at the University of Minnesota. In 1997, an analysis of engraftment suggested that patients at Duke University (all receiving Neupogen 10 μg/kg/day) had a more rapid neutrophil recovery as compared to patients at the University of Minnesota (none receiving haematopoietic growth factor). Subsequently, all patients at the University of Minnesota received Neupogen 5 μg/kg/day.

Analysis of engraftment in patients transplanted at the University of Minnesota demonstrates a trend towards more rapid neutrophil recovery, but only in univariate analysis. When adjusting for other factors that impact upon neutrophil recovery, such as cell dose and disease, the use of prophylactic growth factor is not significant. As there appears to be no deleterious effect (other than cost), the practice of using Neupogen 5 μg/kg/day until the absolute neutrophil count exceeds 2500/μl remains unchanged. As discussed above for HLA, greater patient numbers are required before making any firm conclusions as to the impact of haematopoietic growth factors.

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Question 1

By October 2001, the Duesseldorf cord blood bank had delivered 148 cord blood units (143 unrelated and five related) to more than 50 different transplant centres worldwide. The patients received a median nucleated cell dose (NC) of $4{\cdot}8 \times 10^7$/kg body weight (range: $0{\cdot}95–43{\cdot}18 \times 10^7$). One-hundred and fourteen patients younger than 15 years of age received a median NC/kg body weight of 6×10^7 (range: $1{\cdot}22 – 43 \times 10^7$), whereas 34 patients older than 15 years of age received $2{\cdot}3 \times 10^7$ NC/kg body weight (range: $0{\cdot}95–6{\cdot}13 \times 10^7$).

In a recent analysis of the recipient cytokine genotypes for tumour necrosis factor-α (TNF-α) and interleukin-10 (IL-10) and correlation with graft versus host disease (GvHD) [1], clinical data were available for 128 unrelated cord blood transplants; however, all patients receiving expanded cord blood, multicord blood transplants or cord blood transplants as a rescue of non-engraftment after a previous allotransplant or with an incomplete follow-up at 3 months, were excluded.

The 115 patients (90 children and 25 adults) with a complete follow-up were transplanted with unrelated cord blood between October 1995 and May 2000 in 18 countries and at 57 transplant centres. The median age of the patients was 6·3 years (25–75th percentiles: 1·6–13·2), individual ages ranging from 0·2 to 52·9 years. The cord blood transplants were provided by the cord blood banks in Duesseldorf ($n = 79$), Barcelona ($n = 23$) and Milan ($n = 13$). The majority of patients ($n = 83$; 72%) had malignancies (acute lymphatic leukaemia (ALL), acute myeloid leukaemia (AML), myelodysplastic syndrome (MDS), chronic myeloid leukaemia (CML), non-Hodgkin lymphoma (NHL), Hodgkin's disease, lymphohystiocytic disorders), 24 had inborn errors and eight had bone marrow failure syndromes.

In this cohort of 115 patients, the 1-year survival was dependent on the number of nucleated cells infused > $3{\cdot}7 \times 10^7$ NC/kg body weight ($P = 0{\cdot}01$), as determined by the Kaplan–Meier method and compared using the log-rank test. Statistical analysis was carried out using the SAS software package (SAS Inc., Cary, NC).

The data obtained here are consistent with the data obtained by Rocha *et al.* [2] and Rubinstein *et al.* [3]. However, in addition to the NC, the age of the patient and the recipient diagnosis are important parameters for disease-free survival, as shown by Rocha *et al.* [2] and Rubinstein *et al.* [3].

Question 2

As a major maternal cell contamination of the cord blood graft could contribute to GvHD after cord blood transplantation, all cord blood samples revealing additional alleles in human leucocyte antigen (HLA) serology for HLA-class I or in polymerase chain reaction-sequence specific priming (PCR–SSP) for the HLA-DRβ exon 2 allele, are excluded from cord blood banking. As the sensitivity of the PCR–SSP is high (at detection levels of one in 1000–10 000 cells), leakage from maternal to fetal blood or collection-related problems are detectable by this method [4].

However, with a very sensitive method (a detection level of one in 100 000 cells), maternal contamination in almost each cord blood sample can be detected. Nevertheless, the presence of maternal cells in such low numbers has no apparent effect on GvHD. The presence of maternal cells in cord blood and fetal cells in the maternal blood can be explained by a normal process of materno–fetal transmission occurring during pregnancy.

Other criteria for excluding a cord blood unit are the medical history of the mother and/or the family. These exclusion criteria are discussed in refs [5–7].

According to the NETCORD-FAHCT standards [7], cord blood units must be cryopreserved within 48 h of delivery. In compliance with the German guidelines, cord blood units containing $< 5 \times 10^8$ NC after volume reduction and before cryopreservation, do not qualify for cord blood banking. The maternal blood obtained 48 h before or after delivery must be negative (as tested by commercially available enzyme immunoassays) for human immunodeficiency viruses (HIV)-1 and -2, hepatitis B and C viruses, human T-cell lymphotrophic viruses (HTLV) 1 and 2, and Lues.

PCR testing for cytomegalovirus (CMV) DNA must be carried out on cord blood samples when the mother is positive for CMV immunoglobulin (Ig)G and IgM. In addition, each cord blood sample is tested for hepatitis C virus and parvovirus B19 by PCR. All cord blood units that test positive by PCR are discarded. Before release to a transplant centre, each cord blood unit is tested for hepatitis B virus and HIV by PCR. The immediate exclusion of any cord blood product contaminated with a bacterial or virological infectious agent is mandatory in Duesseldorf, in full compliance with the regulations defined jointly between the scientific advisory board of the Bundesärztekammer and the Paul-Ehrlich-Institute, Germany's central regulatory agency for blood components.

Question 3

The Duesseldorf cord blood bank has a regular, but not obligatory, programme to evaluate the mother and the baby some time after the CB donation. When a cord blood unit has been selected for transplantation, the mother is always contacted

and information on the development and health of the child is obtained.

Question 4

As published in 1999 by Koegler *et al.* [8], in the absence of a marrow donor, one cord blood transplantation of two components (one *ex vivo* expanded, one unmanipulated), both originating from the haploidentical brother's (five of six-antigen match) cord blood, was performed in Duesseldorf in a 2-year-old-girl with chronic ALL, central nervous system (CNS) relapse and blast cells in the bone marrow, at the beginning of the cytoreduction protocol before transplantation. One-eighth of the total cord blood was selected for $CD34^+$ cells, applying the CliniMACS system and expanded in the presence of granulocyte colony-stimulating factor (G-CSF), TPO and Flt3-l in 10% autologous cord blood plasma and X-VIVO 10 medium on day 10 prior to transplantation. The expanded cell population (mainly myelomonocytic, granulocytic cells as well as megakaryocytes) was infused together with the unseparated cord blood on day 0. The patient developed a rapid engraftment of leucocytes, T cells and natural killer (NK) cells, did not develop acute or chronic GvHD and is currently disease free almost 4 years after transplantation.

Presently, we do not apply *in vitro* expansion of progenitor cells systematically, because the majority of the cord blood units provided by the Duesseldorf cord blood bank are transplanted in different countries. The frequency of cord blood transplantation in Germany is presently too low to allow phase I/II studies to be carried out. In addition, in such a study, the use of a marker gene introduced at the start of the *ex vivo* expansion protocol would be necessary in order to follow the fate of the expanded cells in the patients and objectively assess the contribution of these *ex vivo* expanded cells to the phases of short- and long-term haematopoietic engraftment and immune reconstitution.

Question 5

Thus far, the Duesseldorf cord blood bank does not routinely accept autologous donations. Until October 2001 about 200 allogeneic directed cord blood donations of siblings with a clinical indication have been characterized and banked. Until now, five patients (ALL, ALL, amegakaryocytic thrombocytopenia, CML, thalassaemia) have been transplanted with the cord blood of a sibling. Directed cord blood banking is performed only upon written request of the physician responsible for the paediatric patient at risk, or from the respective transplant centre.

Question 6

(a) Rocha *et al.* [9], on behalf of the EUROCORD registry, recently published a retrospective analysis comparing the outcomes after HLA-identical sibling cord blood and bone marrow transplants in children. The same group published the comparison of outcomes after unrelated bone marrow and cord blood transplants in children with acute leukaemia [2]. In both comparative studies, the neutrophil and platelet recovery were significantly delayed in recipients of cord blood compared to bone marrow grafts. Neutrophil and platelet recoveries were always correlated with the nucleated cell dose in the total cord blood unit.

(b) Rocha *et al.* [2] found, in a multicentre analysis, that early transplant-related mortality (TRM) was higher in unrelated cord blood transplant recipients than in unrelated bone marrow transplant recipients; however, Barker *et al.* [10], in a matched-pair single-centre analysis, found that early TRM was similar between unrelated cord blood and bone marrow transplant recipients.

(c) Both acute and chronic GvHD are reduced after HLA-identical sibling and one to four-antigen mismatched unrelated cord blood transplants compared with HLA-identical sibling and unrelated bone marrow transplants, respectively [2,9,10].

(d) Rocha *et al.* [2] and Barker *et al.* [10] found that overall survival was similar between HLA-mismatched unrelated cord blood transplants and unrelated bone marrow transplants in children.

(e) The probability of survival of cord blood transplant recipients was decreased and early TRM increased when three or more HLA disparities (defined by HLA-A and -B serology, and HLA-DRB1 allelic typing) were transplanted. However the number of patients receiving three or four HLA-mismatched cord blood units was too small to allow definitive interpretation. Thirty-two of 507 patients who were registered in the Eurocord registry for a, unrelated cord blood transplant, received a three HLA-mismatched umbilical cord blood. The estimated 100 day-TRM was 50% and the 2-year survival 29%. Rubinstein *et al.* [3] reported that patients receiving cord blood with three or more HLA disparities ($n = 40$) had a 1-year TRM of $\approx 70\%$.

(f) Rocha *et al.* [2] compared children with acute leukaemia receiving either an unrelated bone marrow or a cord blood stem cell transplant. After statistical adjustment of patients and disease characteristics, the relapse rate was not increased after cord blood transplants during the first 3 months after transplantation compared to non-T-cell-depleted bone marrow transplantation.

Question 7

The Duesseldorf cord blood bank has so far provided cord blood for 34 patients (with the majority in advanced stages of disease) older than 15 years (median age 24 years; range: 15–48 years). The patients received $2{\cdot}3 \times 10^7$ NC/kg body weight (range: $0{\cdot}95 - 6{\cdot}13 \times 10^7$). For 29 adult patients a clinical follow-up is available; it is too early to evaluate the remaining five. The present survival rate of the 29 adult patients is 31% (median follow-up time for survivors is 708 days; range: 60–1072 days). For this small cohort of patients, the disease-free survival is identical to data published recently [11–13].

Laughlin *et al.* [11] presented the results of transplantation of HLA-mismatched cord blood in 68 adults with life-threatening haematological disorders. In these patients, the recovery of neutrophils was 90%; 26% were alive and disease free at a median follow-up time of 22 months. The Eurocord group [12,13] reported similar results on 149 adults who underwent unrelated cord blood transplantation; however, the TRM at 180 days was 56% in adults compared to 32% in children enrolled in the same study. These deaths could be correlated with the number of nucleated cells in the graft [12,13]. Patients who received no more than 1×10^7 NC/kg had a 75% probability of death, whereas recipients of at least 3×10^7 NC/kg had a 30% probability of death [12,13].

Rocha *et al.* [14] analysed 108 adults with haematological malignancies, mainly acute and chronic leukaemia transplanted with an unrelated cord blood unit. The median age was 26 years (range: 15–53 years), median weight 60 kg (range: 35–110 kg) and median follow-up time 20 months (range: 0·6–60 months). Nineteen per cent of the patients had previously received an autologous bone marrow transplant. Six patients received an HLA-identical unit and 102 an HLA-mismatched unit with one HLA difference (38 patients), two HLA differences (51 patients) or three HLA differences (13 patients). The median number of nucleated cells infused was $1{\cdot}7 \times 10^7$/kg (range: 0·2–6). The neutrophil recovery on day 60 was 81%, and platelet recovery on day 180 was 65%. Twenty-four patients died early (between days 4 and 57); 15 did not engraft. The median time for neutrophil recovery was 32 days (range: 13–57 days) and that for platelets (> 20 000/mm^3) was 129 days (range: 26–176 days). The number of NC > $1{\cdot}7 \times 10^7$/kg was an important factor influencing neutrophil and platelet recovery (P = 0·01). Acute GvHD (> grade II) was observed in 44 patients (grade II = 16, grade III = 12 and grade IV = 15).

The occurrence of grade III–IV GvHD did not reveal an influence of the number of HLA disparities (P = 0·12) in this limited patient cohort. Chronic GvHD was observed in 15 of 58 patients at risk. The Kaplan–Meier estimate for TRM on day 100 was 54%. In a univariate analysis the following factors increased 100-day TRM: poor risk disease status at cord blood transplantation (P = 0·016); < $1{\cdot}7 \times 10^7$ NC/kg (P = 0·03), and a cord blood transplantation performed before January 1998 (P = 0·01). Patients transplanted before January 1998 received cord blood units containing a lower number of nucleated cells (P = 0·03) and a greater number of HLA disparities (P = 0·001) compared to patients transplanted after January 1998. The 1-year survival was 27% and event-free survival (EFS) was 21%. Favourable factors for survival were > 1×10^7 NC/kg (P = 0·014) and the good-risk status of the patient (P = 0·007).

In conclusion, these results suggest that cord blood is an optional source of stem cells in adult patients with malignancies who lack a matched unrelated bone marrow donor.

Question 8

Rubinstein *et al.* [3] and the Eurocord group have shown that the number of HLA disparities is associated with the speed and probability of neutrophil and platelet engraftment. Rubinstein *et al.* [3] showed a 69% probability of neutrophil recovery for those patients receiving a three HLA-mismatched cord blood transplant (n = 40).

Gluckman *et al.* [13] reported a 55% probability of neutrophil recovery in 32 patients receiving an unrelated cord blood transplant with three HLA differences compared to 82% for an HLA-matched unrelated cord blood transplant, 85% for those with one HLA difference and 76% for those with two HLA differences.

Question 9

To date, in the largest series of unrelated cord blood transplants analysed, the number of HLA differences did not influence the occurrence and severity of acute GvHD. Only in patients with six out of six antigen matches was the frequency of grade III–IV GvHD clearly lower than in other patients.

Question 10

The use of prophylactic haematopoietic growth factors (i.e. day of onset between 0 and 7) did not markedly influence the probability of neutrophil recovery and TRM in a retrospective analysis of the Eurocord registry. However, the use of growth factors shortened the period of aplasia after transplantation (data not published). Only a randomized prospective analysis will be able to answer this question definitively.

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Question 1

We agree that the most important factor in predicting a positive outcome for a transplant is the number of nucleated cells infused, as this is clearly shown by a number of studies, in particular the recent analysis from the Eurocord Registry [1].

Question 2

In our programme, maternal DNA can be detected at the time of banking, when human leucocyte antigen (HLA) typing is performed at low resolution (reverse dot blotting), and/or at a later time-point, when high resolution DRB1 typing is carried out with sequencing-based typing (SBT) for the final confirmation of matching, soon before transplantation. When contamination is detected [2], we inform the clinician of the presence of maternal DNA in the unit and leave the decision on the actual use of the unit to him/her. We adopted this policy because there is no consensus on the impact of the presence of maternal DNA in cord blood on the outcome of a cord blood transplant and because a unit containing maternal DNA may represent the only chance for a specific patient. We also offer the clinicians the possibility to expand the evaluation of the presence of maternal DNA in the unit by performing microsatellite analysis with capillary electrophoresis with a genetic analyser. This method is more informative (10 loci analysed) than HLA typing and shows a sensitivity of 1%.

Our acceptance criteria for cord blood banking are shown below:

- absence of family history of inherited diseases, risk behaviour in the parents, history of positive serological tests on the parents, delivery before 34 weeks of gestation, congenital abnormalities in the newborn, fever in the mother (> 38 °C) the day before or after delivery, fetal distress;
- availability of mother's written informed consent to cord blood collection, banking, testing at donation and at 6 months, electronic data storage and cord blood unit release;
- freezing within 36 h of cord blood collection;
- correct cord blood unit and samples identification;
- cord blood volume > 60 ml without anticoagulant;
- total nucleated cells > 800×10^6 after volume reduction of the cord blood unit;
- negative serology on mother's serum: hepatitis B surface antigen (HBsAg); syphilis; antibodies to human immunodeficiency viruses (HIV)-1 and -2; antibodies to hepatitis C virus (HCV); and antibodies to human T-cell lymphotrophic viruses (HTLV) I and II; and
- absence of bacterial contamination with recognized pathogens.

Question 3

We routinely evaluate mothers and babies 6 months after delivery. This programme has been in place since 1996. The main outcomes, which have been evaluated in a study reported in detail previously [3], were as follows: of the 2450 mothers enrolled into the study, 2315 (94·5%) attended the bank in agreement with the programme, four promised to attend, 95 were not traced, 26 declined the invitation and 10 were unable to attend. Of the 135 mothers who were not

traced back, 29 (21·4%) belonged to non-Caucasian ethnic groups. The average contact time with each mother was ≈ 20 min. As regards serology, one indeterminate anti-HCV seroconversion (c22) was detected. As regards the babies, one case of congenital urinary malformation not known at delivery, one of protein C deficiency, one of phenylketonuria, one of mucoviscidosis, and one of 10q chromosomal abnormality, were reported. All these units were discarded.

Question 4

We developed a serum-free and stroma-free expansion protocol using a cocktail containing: thrombopoietin, Flt3 ligand, interleukin (IL)-6 and IL-11. When this protocol was used at a small-scale laboratory level, a 2–3 log expansion of nucleated cells was obtained [4]. We are currently scaling up this procedure under clinical grade conditions and using clinical grade reagents. This will be performed in two BL3 laboratories built in our institution, which were recently accredited by the Italian Ministry of Health (code MI/IC/IMP.II/00-02). At present this is in the experimental phase.

Question 5

We do not accept cord blood donations for autologous use if the donor is not affected by a condition for which it can be expected that autologous haemopoietic progenitors could be beneficial. As regards the allogeneic-directed donations, our criteria are as follows: we do not accept requests sent directly by patients or their families; rather, the request to store a cord blood unit for allogeneic-related transplantation must be forwarded by a transplant center. The donor must be family related (generally a brother or a sister), and the patient must suffer from conditions that may be treated with cord blood transplantation.

Question 6

We participated in a Eurocord study recently carried out to compare the outcomes in children with acute leukaemia who were transplanted with cord blood, unmanipulated bone marrow or T-cell-depleted bone marrow [1]. When bone marrow was taken as a reference, cord blood recipients showed delayed neutrophil and platelet recoveries, higher transplant-related mortality in the first 100 days and a lower occurrence of acute and chronic graft-versus-host disease (GvHD). Kaplan–Meier estimates of event-free survivals at 2 years in cord blood and bone marrow recipients were 31% (95% CI: 21–41) and 43% (95% CI: 37–49), respectively. Among the patients surviving for longer than 100 days, the risk of death was not significantly increased in cord blood versus bone marrow recipients ($P = 0{\cdot}55$). No correlation was found between the number of HLA mismatches and the outcome of cord blood transplantation. Finally, no difference was detected between risks of relapse in cord blood and bone marrow recipients.

Question 7

To date we have released a total of 133 cord blood units for unrelated transplantation. Of these, 40 (30%) have been used in adult patients (> 15 years of age), who were transplanted in 20 transplant centers. The outcome analysis of these patients is carried out in co-operation with the Eurocord Registry in Paris. At the time of this writing, a formal analysis of our adult recipients has not been carried out because the number is too small to achieve sufficient statistical power. A recent analysis performed by other investigators on 68 adult cord blood recipients, with a median age of 31·4 years and body weight of 69·2 kg, showed that 18 patients (26%) were alive and disease-free 40 months after transplantation [5].

Question 8

Although a clear (i.e. statistically significant) HLA effect has not been demonstrated [1,5] in regard to transplant success, this may be a result of the relatively small number of patients so far available. In one series, a statistically significant association of transplant success with histocompatibility matching grades was reported [6]. Hence, we consider that HLA could be an important factor for both engraftment and GvHD.

Question 9

In the study on adult cord blood recipients that was published by Laughlin *et al.* [5], there was no statistically significant association between the grade of acute GvHD and the degree of HLA mismatching ($P = 0{\cdot}70$) or mismatching in HLA class II alleles ($P = 0{\cdot}68$).

Question 10

No. We have no experience with the use of haematopoietic growth factors to improve the speed of engraftment.

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Question 1
Yes, we agree that the most important factor in predicting a positive outcome for a transplant is the number of nuclear cells infused.

Question 2
No. Our criteria for rejecting a cord blood unit are described below.

Exclusion criteria. Mother.
Absolute criteria:
- legal minor (< 18 years of age);
- inability to understand the given information;
- consanguinity with the father (up to first-degree cousins);
- other infant stillborn or deceased early;
- transfusion of cellular blood products or fresh-frozen plasma;
- organ transplant;
- treatment with pituitary extracts, i.e. growth hormone before 1989;
- autoimmune disease of genetic or hereditary origin;
- history of cancer;
- transmissible maternal infection [hepatitis B or C virus, acquired immune deficiency syndrome (AIDS), syphilis, human T-cell lymphotrophic virus (HTLV), malaria];
- 'at risk' behaviour (prostitution, drug addiction, multiple partners or homosexuality);
- potentially mutagenic drug intake during pregnancy;
- active infection with cytomegalovirus (CMV) or toxoplasmosis during pregnancy;
- gestational age below 35 weeks;
- rupture of membranes for more than 12 h or maternal fever above 38 °C;
- twin pregnancy.

Relative criteria. If the following criteria are present, it only implies additional testing.

The control of viral serology of the mother after 3 months is indispensible to validate the unit if:
- change of partner within the last 6 months;
- residence or travel to a high-risk zone regarding human immunodeficiency virus (HIV) infection within the last 6 months;
- surgical operation or endoscopic procedure within the last 6 months;

Malaria serology control must be performed if:
- personal history of malaria;
- residence or travel to a high-risk zone regarding malaria infection;

Question 3
Yes. A statement is requested on the medical history and examination of the baby from the paediatrician or general practitioner and a serology control after 3 months.

Question 4
In vitro expansion of progenitor cells is not applied at present.

Question 5
Autologous donations are not allowed. Allogeneic donations are allowed from a family with a diseased child.

Question 6
(a) Yes, neutrophil and platelet recovery occurs later than after bone marrow transplantation.

(b) Yes early post-transplant mortality is higher.

(c) Yes, the occurrence of acute or chronic GvHD is lower, even in the presence of major HLA-class I and/or class II mismatches.

(d) Yes, the long-term outcome is not worse than after bone marrow tranplantation.

(e) No. There is no correlation between the number of HLA mismatches and survival.

(f) No. There is no difference in leukaemia relapse after cord blood or bone marrow transplantation.

Question 7
We have used cord blood transplantation in three adults. One is alive and well, one is alive but has experienced rejection, and the third experienced poor engraftment and died as a result of infection.

Question 8
No, we do not consider that HLA incompatibility unfavourably influences engraftment.

Question 9
Yes, we think that HLA incompatibility is related to the severity of GvHD.

Question 10
We use granulocyte colony-stimulating factor (G-CSF) to improve the speed of engraftment.

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Question 1
Yes, the majority of published results indicate that engraftment, survival, neutrophil and platelet recovery in patients transplanted with cord blood correlate with the nucleated cell dose/kg infused. It has been reported that an infused cell dose of $> 3{\cdot}7 \times 10^7$/kg is the most important factor affecting neutrophil engraftment [1]. More recently it has been reported that the colony-forming cell (CFC) content of the cord blood unit is a better predictor of transplant outcome [2], but these results need to be confirmed, particularly in view of the lack of standardization regarding this test. It is not yet clear to what extent these two factors are related to the degree of human leucocyte antigen (HLA) mismatches between the donor and recipient.

Question 2
At the London Cord Blood Bank (LCBB), we do not routinely assess the presence of maternal contamination in the units. However, we have HLA typed more than 5000 cord blood units using polymerase chain reaction (PCR) DNA techniques and thus far have had no evidence of the presence of maternal DNA in the samples (as indicated by the presence of additional maternal HLA alleles other than those expected from the inherited haplotype). It should be mentioned that although the presence of maternal (mature and progenitor) cells has been reported in approximately 20% of cord blood collections, it is not yet clear whether these cells play any role in the outcome of the transplant by either contributing to the graft-versus-host disease (GvHD) effect by modifying the recipient T-cell repertoire, or by regulating the induction of tolerance.

Therefore, at present, maternal cell contamination is not a criterion for the LCBB to reject CB donations. However, cord blood units are excluded for the following reasons:

- collected volume of < 40 ml;
- total nucleated cell count of $< 4 \times 10^8$ after processing;
- total nucleated cell recovery of < 70% after processing;
- missing samples and results;
- repeat reactive results for the following mandatory infectious disease markers: antibodies to hepatitis B surface antigen (HBsAg); antibodies to human immunodeficiency viruses (HIV)-1 and -2; and antibodies to hepatitis C virus (HCV);
- an abnormal blood film report;
- failure to obtain informed written consent from the mother for the voluntary donation, storage and use of the cord blood unit and for testing infectious disease markers;
- when a donor mother is excluded for medical reasons – these include diseases where an infectious aetiology is considered possible, or where there is a significant risk of genetic or other disease transmission;
- where the pregnancy is a result of *in vitro* fertilization (IVF) using a donated ovum or sperm;
- when the donor mother has received a blood transfusion within the last year;
- where the donor mother has had tattoos, application of semi-permanent make-up, acupuncture (other than by approved practitioners), body piercing, or accidental needlestick injury within the last year.

Question 3
All donor mothers are contacted by telephone 8 weeks after delivery by a trained member of the Cord Blood Bank staff. The interview is conducted using a questionnaire designed to determine the postnatal health of both mother and baby. Answers to the questions may lead to correspondence, with the mother's permission, with the family's general practitioner or other health professionals. Mothers are also invited to report any medical complications occurring after donation.

A search of the Cytogenetics and Malformation Regional Registry is initiated at the time a donation is selected as a potential match for a patient.

Question 4
We are not currently involved in any programme to expand HSC or progenitor cells.

Question 5
Allogeneic directed donations are only accepted if requested by the transplant clinician treating the patient who may require a transplant. Referrals are accepted only in the following circumstances:

- where the intended recipient is younger than 16 years of age or whose weight is commensurate with that age;
- where the cord blood collection is intended as a vehicle for gene therapy for the index donor infant, if affected with a genetic disease amenable or potentially amenable to gene therapy in the future; or
- for future children, of the same parents, at risk of a specific genetic disease treatable by bone marrow transplantation where the index donor is not affected by that genetic disorder.

Collections will not be accepted from mothers with positive mandatory marker screening tests. If the baby should be delivered before 34 weeks of gestation, the collection will not proceed.

The Cord Blood Bank co-ordinates the arrangements for collection by approaching the relevant obstetrician and donor mother to gain written consent for the collection to be made. The Cord Blood Bank staff closely liase with the midwifery staff at the delivery hospital, providing training and all the necessary equipment for the *in utero* cord blood collection.

Question 6

(a) One of the main differences in the overall results obtained using cord blood is the delayed engraftment and speed of myeloid and platelet recovery, which correlates strongly with the TNC infused [3]. The number of HLA mismatches seem to have an important influence on the myeloid and platelet recovery, but there appears to be no correlation between the number of HLA mismatches and overall outcome of the transplant [4].

(b) Early mortality rates in unrelated cord blood transplantation and HLA-matched bone marrow transplantation have been shown to be similar [5]. Our own analyses showed that only two patients died before day 60 and that 13 (43%) died before day 100.

(c) Both acute and chronic GvHD have been shown to be decreased in cord blood transplantation, even in the presence of HLA disparity. In our analysis of the survival data on 30 patients transplanted with units provided by the LCBB, the Kaplan–Meier estimate of acute GHvD at 100 days was 39% (grade 0–I = 63%; grade II = 17%; grade III = 10%; and grade IV = 10%). The Kaplan–Meier estimate for chronic GvHD at 2 years was 15%. Unfortunately, there were insufficient data to assess the impact of HLA mismatches on the development of either acute or chronic GvHD.

(d) No data are available yet from our study in regard to long-term outcome, but the articles published by Rocha *et al.* [4] and Barker *et al.* [5] have shown that survival following transplantation with umbilical cord blood from an unrelated donor is comparable to the survival obtained using an HLA-matched unrelated bone marrow donor.

(e) Most of the published studies have shown a direct correlation between the number of HLA mismatches and survival. In our series the correlation between HLA mismatches and survival is as follows: 0 HLA mismatches, 43% survival; 1 mismatch, 40% survival; and two mismatches, 36% survival. There was only one patient with three mismatches who is still alive.

(f) Published data indicate that there is no difference in the relapse rate using umbilical cord blood or bone marrow except if patients are transplanted when in an advance stage of the disease [4]. No data are available yet from our study.

Question 7

In our analysis the effect of recipient age was not statistically significant because 76% of the patients younger than 15 years of age engrafted compared to 86% of patients older than 15 years ($P = 0{\cdot}44$). However, we did find a significant effect of patient age on the overall survival, where 54% of patients < 15 years of age are still alive compared with only 11% of patients ≥ 15 years ($P = 0{\cdot}005$). The analysis of patient weight (< 30 kg or > 30 kg) showed no difference with respect to the percentage of patients engrafting or death.

However, the results published by Laughlin *et al.* showed that cord blood transplantation was successful in restoring haematopoiesis in adults [6].

Question 8

In our study there was no association between the degree of HLA incompatibility and engraftment. It is possible that these discrepancies may be explained by the small number of cases analysed in our study.

Question 9

Although the results from the large series of patients analysed have shown that HLA disparity has no major effect in the development of acute GvHD [4], the degree HLA incompatibility, e.g. one versus two versus three mismatches, may still have a major effect on the overall reduced GvHD observed following cord blood transplantation. However, it should be noted that the effect of HLA mismatches on the outcome of cord blood transplantation has been difficult to evaluate owing to the different techniques (DNA versus serology) and degree of resolution at which patients and donors are typed at individual centres.

Question 10

No.

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M. J. Laughlin

Question 1

I agree that the most important factor in predicting a positive outcome after umbilical cord blood transplantation is the number of nucleated cells infused. In our analyses of transplant outcomes in 68 adults [1] with haematological disorders, the kinetics of neutrophil engraftment was noted to correlate with the number of cells in the graft before freezing ($P = 0{\cdot}003$). We did not observe a statistically significant association between graft failure and receipt of umbilical cord blood grafts with increasing human leucocyte antigen (HLA) mismatching to the patient (HLA 5/6 and 6/6 match versus 4/6, versus 3/6) ($P = 0{\cdot}5$), or receipt of umbilical cord blood grafts mismatched to the patient at HLA class II alleles ($P = 0{\cdot}2$). These findings in adult umbilical cord blood transplant recipients parallel previous reports. The clinical study undertaken by Dr Rubinstein *et al.* [2], reported that the kinetics of donor-derived myeloid engraftment was associated with the leucocyte content of the umbilical cord blood graft. In addition, Dr Gluckman [3] noted that neutrophil recovery was observed in 94% of the patients who received a higher cell dose (37 million or more nucleated cells/kg) from unrelated umbilical cord blood donors. Moreover, Dr Kurtzberg [4] stated that the number of nucleated cells infused/kg of the recipient's weight correlated with the rate of myeloid engraftment ($P = 0{\cdot}002$). These clinical reports therefore identify nucleated cell dose as an important factor in predicting donor engraftment after umbilical cord blood transplantation.

Question 2

We do not consider the detection of extra maternal HLA antigens a criterion for rejecting a cord blood sample. The criteria we use in accepting or rejecting an umbilical cord blood graft involves a process that first includes preliminary searches of umbilical cord blood banks using the patient's HLA phenotype, based on serological or molecular typing for class I HLA-A and -B antigens and low-resolution DNA typing for class II HLA alleles. High-resolution molecular typing for HLA-DRB1 alleles is then performed as confirmatory typing. Preferred units are those matched at 4/6 HLA loci or better and containing a minimum prefreeze cell dose of $1{\cdot}5 \times 10^7$ nucleated cells/kg recipient body weight. In some cases, a lesser-matched graft with a higher number of nucleated cells is selected over a more closely matched graft with fewer cells. Our reasons for specifying HLA 4/6 matched grafts is based on our analyses of adults receiving HLA 3/6 matched grafts who demonstrate inferior survival. Although a minimum prefreeze cell dose of $1{\cdot}5 \times 10^7$ nucleated cells/kg recipient body weight is outlined in our protocols, we will continue to search, or consider enrolment on our multiple unit protocol or *ex vivo* expansion, because cell dose is correlated strongly with transplant outcomes, including engraftment and survival.

Question 3

Our transplant programme does not currently bank unrelated umbilical cord blood for clinical transplantation.

Question 4

We do not currently apply *in vitro* expansion of umbilical cord blood progenitor cells in adult recipients. In an attempt to shorten the time interval to haematopoietic recovery after umbilical cord blood transplantation, studies by several investigators have been undertaken to expand umbilical cord

blood grafts in cytokines *ex vivo* prior to infusion [5,6]. These clinical trials have not demonstrated improved kinetics of engraftment in patients receiving culture-expanded grafts. Concerns are raised with attempted cellular expansion of umbilical cord blood in cytokine-based cultures during which proliferation and differentiation of early self-replicative stem cells may adversely affect stem cell self-renewal, amplification and homing. Moreover, these ongoing preclinical and phase I clinical studies incorporating CD34 selection or hydrocortisone in umbilical cord blood cultures raise further questions related to the role of accessory lymphoid populations in facilitating allogeneic engraftment of *ex vivo*-expanded umbilical cord blood grafts, knowing that T depletion of allogeneic grafts is associated with higher graft failure rates.

Question 5

Our criteria for collecting related autologous or allogeneic directed donations includes any adult or child carrying a diagnosis, generally a haematological disorder, for which stem cell transplantation is a known effective treatment.

Question 6

The focus of our umbilical cord blood transplant programme is in adult patients. We observe delayed neutrophil and platelet recovery in adults infused with umbilical cord blood grafts compared with related or unrelated mobilized peripheral blood stem cell grafts from adult donors. Early post-transplantation mortality is higher in umbilical cord blood recipients owing to infection and/or organ toxicity. Despite the fact that the majority of our adult patients receive umbilical cord blood grafts mismatched at more than one HLA locus, the incidence of severe acute graft-versus-host disease (GvHD) ranges from 18–22%, which is a lower incidence than that observed in adults grafted with HLA-matched unrelated grafts from adult donors. Adult umbilical cord blood recipients in our experience exhibit lower rates of chronic GvHD (≈ 30%), and the majority of these umbilical cord blood patients demonstrate limited, non-progressive GvHD. A measure of the long-term outcome in umbilical cord blood adult recipients is event-free survival (EFS), ranging from 28–32%, which parallels transplant outcomes in patients receiving HLA-matched unrelated adult donor grafts who are similar in age and disease stage. The majority of our adult umbilical cord blood patients have returned to full-time employment and do not experience significant late complications beyond 1 year post-transplant. Adult patients infused with HLA 3/6 matched grafts in our experience have demonstrated inferior transplant outcomes, but we have not observed significant differences in rates of engraftment or survival when comparing patients infused with HLA 4/6 versus 5/6 or 6/6 matched grafts. The rates of relapse after HLA mismatched umbilical cord blood transplant in adults is ≈ 15% and there appears to be strong graft-versus-leukaemia immune effects exerted by umbilical cord blood grafts, perhaps related to HLA class I disparity. We have not, however, performed a direct comparison of relapse rates with that of our patients infused with related or unrelated HLA-matched grafts from adult donors.

Question 7

A major focus of our transplant programme is the study of HLA-mismatched unrelated umbilical cord blood transplantation in adults with haematological disorders. The majority of these patients are infused with umbilical cord blood grafts disparate at two HLA antigens. Primary graft failure is observed in 10% of these patients and neutrophil engraftment is delayed, occurring, on average, 27 days after transplantation. Platelet and red cell engraftment are also delayed, occurring, on average, 2 months post-transplant. We observe acute GvHD of grades II–IV in 60% of these patients, and 20% exhibit more severe grades III–IV acute GvHD. Chronic GvHD is manifested in approximately one-third of patients surviving beyond day 100 and is extensive/progressive in only a few. We have followed these patients for up to 5 years and survival is 25%, reflective of high-risk patients enrolled on early phase I trials and high peritransplant mortality related to delayed engraftment. The immunological naivety of umbilical cord blood accessory cells allows allogeneic engraftment without requirement of T depletion and, despite infusion of HLA disparate grafts, an acceptable incidence of severe acute GvHD and chronic GvHD is observed.

Question 8

In reviewing data from our transplant programme and the published literature, I feel that there is a threshold effect for HLA matching in unrelated umbilical cord blood transplant. The unique immunological features of umbilical cord blood graft lymphocytes allow engraftment despite greater HLA disparity between donor and recipient. The minimum matching that ensures acceptable transplant outcomes after unrelated umbilical cord blood transplant is HLA 4/6 loci, using criteria of serological typing at class I loci and low-resolution matching at class II loci. Importantly, patients undergoing unrelated umbilical cord blood transplantation have generally received preparative regimens of increased intensity to facilitate engraftment, which have also resulted in higher treatment-related mortality, particularly in adult recipients [1]. HLA disparity in conventional related and unrelated blood and marrow grafting can result in graft rejection and GvHD. Improved clinical outcomes have been facilitated by the development of accurate, reproducible high-resolution DNA-based HLA typing methods. The further application of these DNA-based HLA typing methods to class I typing would be expected to further improve successful engraftment after unrelated umbilical cord blood transplantation.

Question 9

The data do not support the concept that HLA incompatibility is correlated to the severity of GvHD. In our analyses of 68 adults transplanted with single-unit umbilical cord blood after full myeloablative conditioning, of 54 engrafted patients surviving > 28 days, 22 had grade 0–I acute GvHD with single organ involvement (skin), 22 had grade II, seven patients had grade III, and four had grade IV acute GvHD. This report summarized the composite pilot experience at five centres utilizing HLA-mismatched unrelated umbilical cord blood as an alternative allogeneic stem cell source in high-risk adult patients. The actuarial probabilities of grades II–IV and III–IV acute GvHD by 100 days post-transplant in this series were 0·60 (CI: 0·49, 0·71) and 0·2 (CI: 0·11, 0·29), respectively. There was no association between the grade of acute GvHD and HLA disparity (P = 0·7) or HLA class II disparity (P = 0·7), the presence of pretransplantation cytomegalovirus (CMV) seropositivity in the recipient (P = 0·3), or administration of total body irradiation (TBI) versus chemotherapy-based conditioning (P = 0·7).

Umbilical cord blood T lymphocytes are typically $CD45RA^+$ and express low levels of activation markers, both of which are consistent with a naïve T helper 0 (Th 0) phenotype [7]. Therefore, the reduced GvHD summarized in clinical reports after umbilical cord blood transplantation may be related to these *in vitro* observations that immunologically competent cells contained in an umbilical cord blood graft, although capable of recognizing non-inherited antigens, do not elicit the normal cascade of events to expand these alloreactive lymphocytes. This low incidence of severe acute GvHD in adult umbilical cord blood recipients contrasts favourably with the 35–55% incidence of grade III/IV acute GvHD reported in patients receiving HLA-matched bone marrow from unrelated donors using standard cyclosporin and short-course methotrexate for prophylaxis [8,9]. In addition, the probability of chronic GvHD in this series was 37% and was limited stage in all but one patient. In comparison, patients receiving HLA-matched unrelated donor bone marrow transplants are noted to have chronic GvHD rates ranging from 55–75% in evaluable patients.

Question 10

The majority of our patients received filgrastim (Amgen, Thousand Oaks, CA) 5–10 mg/kg subcutaneously or intravenously, daily from day 0 until durable neutrophil recovery was achieved. The role of optimal combinations of haematopoietic growth factors administered *in vivo* after umbilical cord blood transplantation is yet to be fully elucidated.

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Question 1

I agree that from available evidence the total number of nucleated cells (TNC) infused is the most important predictor

of clinical outcome. A number of studies have shown a positive correlation between CD34 counts and event-free survival. However, it is unclear from these studies whether the CD34 count is independent of TNC as a predictor of outcome. This centre provides a threshold for banking of 40×10^7 TNC after volume reduction. For possible, future uses, CD34 counts are also measured routinely and clonogenic assays performed on selected samples.

Question 2

We do not test donations for maternal cell human leucocyte antigens (HLA). We can, if required, do this retrospectively on stored samples and it is partly to facilitate this that a maternal sample is also stored for future testing. I am unaware of evidence that such contamination has any bearing on clinical outcome.

Question 3

We have a programme to evaluate mother and baby after the donation. The donor is asked to provide consent for such assessment, including accessing the medical notes if required. A routine assessment is carried out at 8 weeks by telephone interview with the mother. This enables the collection of feedback on the routine baby check-up which is carried out at around 6 weeks of age. At this time the full family history questionnaire is completed and any queries referred to the medical advisor for further investigation. At the time of selection for transplant any queries are identified and investigated by the medical advisor.

Question 4

We do not employ *in vitro* expansion of progenitor cells.

Question 5

Requests for directed donations must include a request in writing from the potential recipient's medical consultant in addition to the mother. Our current policies only allow directed donations for allogeneic use. In general, where there is even a small possibility of a future stem cell transplant, the request is acceded to. This may, of course, require referral of the mother for delivery to a unit staffed by midwifes trained in cord blood collection.

Questions 6–10

I have no direct clinical involvement in this area and therefore cannot add to experience reported from elsewhere.

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