firm or refute these concerns. We chose to select for WT1 vaccine treatment only those patients with progressing MDS with excess of blasts, because prevention or delay of overt leukemia could outweigh the disadvantage of inducing cytopenia.

Finally it remains a possibility that the entire T-cell response to MDS is a side-show, and we should direct our attention more to myelosuppression by NK cells shown by Chamuleau *et al.*[1] to be strongly and specifically cytotoxic to MDS cells and perhaps in some patients (e.g. non-responders to ATG) responsible for myelosuppression and immune surveillance. In conclusion, the relationship between the immune system, marrow suppression and MDS remains confusing. Comprehensive studies in a large group of patients as performed by Chamuleau *et al.* are critical steps forward in trying to establish a global view of competing mechanisms contributing to the two major outcome determinants of MDS – marrow failure and leukemic progression.

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Cord blood transplantation: state of the art

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Hematopoietic stem cell transplantation (HSCT) can be curative in a large variety of selected malignant and non-malignant diseases. Cord blood is an unlimited source of hematopoietic stem cells for allogeneic hematopoietic stem cell transplant. Umbilical cord blood transplantation (UCBT) has extended the availability of allogeneic HSCT to patients who would not otherwise be eligible for this curative approach. Since the first human cord blood transplant performed twenty years ago,[1] cord blood banks (CBB) have been established for related or unrelated UCBT with more than 400,000 units available and more than 20,000 umbilical cord blood transplants performed in children and in adults. UCB has many theoretical advantages due to the immaturity of newborn cells. UCB hematopoietic progenitors are enriched in primi-

tive stem/*progenitor* cells able to produce *in vivo* long-term repopulating stem cells. The properties of UCB cells should compensate the relatively low number of cells contained in a single umbilical cord blood and, through rapid expansion, reconstitute myeloablated patients. Despite the capacity for cord blood cell expansion, clinical results showed that hematopoietic recovery was delayed after UCBT; engraftment was associated with the number of nucleated and $CD34^+$ cells infused and the number of HLA differences.[2,3] As acute graft-versus-host disease (GVHD) is an early event after allogeneic BMT and is in part triggered by cytokine release, it is reasonable to postulate that UCBT induces less frequent and less severe acute and chronic GVHD than adult HSCT which contain a higher number of activated T-cells. These properties should lead to less stringent criteria for HLA donor-recipient selection. In comparison with other sources of allogeneic HSCT, UCB offers substantial logistic and clinical advantages such as (i) significantly faster availability of banked cryopreserved UCB units, with patients receiving UCB transplantation in a median of 25-36 days earlier than those receiving BM;[4] (ii) extension of the donor pool due to tolerance of 1-2 HLA mismatches out of 6 (higher HLA mismatched is associated with lower probability of engraftment); (iii) lower incidence and severity of GVHD; (iv) lower risk of transmitting infections by latent viruses; (v) lack of donor attrition; (vi) lack of risk to the donor, and finally (vii) higher frequency of rare haplotypes compared to bone marrow registries, since it is easier to target ethnic minorities.

Clinical results of cord blood transplant

In a CIBMTR Eurocord study, comparing pediatric BM and CBT from HLA identical sibling, UCBT was associated with delayed granulocyte and platelet engraftment, reduced acute and chronic GVHD and same survival. This was the first analysis which demonstrated, unambiguously, that GVHD was reduced when CB cells were used instead of BM even when it was provided by children.[5] This first study was the basis for advocating the use of mismatched UCBT and triggered the development of unrelated cord blood banks.

The second step was the demonstration that unrelated CBT could be used in all current indications of allogeneic HSCT including malignant and non-malignant diseases in children and in adults.

In children with malignant diseases, two studies compared the outcome of unrelated UCBT and BMT. Eurocord published a study comparing the outcome of matched unrelated BMT (HLA 6 out of 6) either unmanipulated or T depleted to mismatched UCBT. Results showed that after UCBT, engraftment was delayed, GVHD was reduced similarly to T-cell depleted BMT; relapse was the same as was leukemia free survival.[6] Eapen M *et al*. compared outcomes of 503 children with acute leukemia given an unrelated mismatched UCBT with 282 unrelated BM transplant recipients (116 HLA allele matched 8 out of 8). HLA allele mismatched BM recipients had more acute and chronic GVHD without decreasing leukemia free survival (LFS). Importantly, they found that even using an allele matched BM donor, LFS was not statistically different from one or 2 HLA disparate UCBT and that an HLA matched UCBT recipient had better outcomes compared to HLA allele matched BM recipients. However, an increased transplant related mortality was observed in children transplanted with a low CB cell dose ($<3\times10^7$/kg) and 1 HLA disparate CB graft or in children given a 2 HLA disparate UCBT independently of the cell dose infused. Interestingly, use of 2 HLA mismatched UCBT was associated with lower incidence of relapse.[7]

The same studies were performed in adults with malignancies. The Eurocord study compared adults with acute leukemia receiving either a matched unrelated bone marrow transplant (HLA 6 out of 6) or a mismatched cord blood transplant. Results showed that, despite a delay of engraftment, CBT gave a similar leukemia survival to BMT.[8] In the same issue of the journal CIBMTR and NYCBB showed that, in adults with malignancies, CBT gave the same LFS survival to 1 antigen mismatched UBMT.[9] At the same time, a Japanese study showed that CBT gave better results than MUD.[10] In a meta analysis combining the published studies, 161 children and 316 adults undergoing UCBT (mostly 1 or 2 antigen-mismatched), along with 316 children and 996 adults undergoing UBMT (almost entirely fully matched with the recipient), were analyzed. T-cell depleted UBMT was excluded; where data were available, only fully matched UBMT was used in the analysis. Pooled comparisons of studies of UCBT and UBMT in children found that the incidence of chronic GVHD was lower with UCBT, but the incidence of grade III–IV acute GVHD did not differ. There was no difference in 2-year overall survival in children when studies were pooled. For adults, there was no statistical difference between transplantation-related mortality (TRM) and LFS.[11] Recently, Eurocord and CIBMTR performed a study comparing the outcome of unrelated HLA matched or 1-2 antigens mismatched bone marrow (n=364) or G-CSF mobilized peripheral blood (n=728) to mismatched cord blood transplant (n=148) in adults with acute leukemia. In multivariate analysis, in UCBT, TRM was higher but relapse rate and GVHD were lower resulting in the same LFS compared to the other sources of stem cells (*unpublished results*).[12] The results of these comparative studies and the meta-analysis considered together showed that (i) UCBT is feasible in adults when a cord blood unit contains a higher number of cells and should be considered an option as an allogeneic stem cell source for patients lacking an HLA matched bone marrow donor; (ii) despite increased HLA disparity, UCB from unrelated donors offers sufficiently promising results to matched UBM in adults with hematologic malignancies leading to the conclusion, as in children, that the donor search process for BM and UCB from unrelated donors should be started simultaneously, especially in patients with acute leukemia, where the time factor is crucial.

Cord blood bank development

The progress in the field of umbilical cord blood transplantation is paralleled by the huge interest in establishing and developing cord blood banks worldwide. Today, more than 400,000 cord blood grafts are available in more than 50 cord blood banks *www.bmdw.org*.

These banks play an important role in the process of cord blood transplantation. A survey of the International Bone Marrow Transplant Registry (CIBMTR) estimates that after 1998, 20% of stem cell transplants performed in young patients (<20 years old) are cord blood transplants (IBMTR Newsletter). In Japan, nowadays approximately 50% of HSCT from unrelated donors are being performed with cord blood cells. This development is due to the organization of international registries for outcome data collection named Eurocord *www.eurocord.org* and CIBMTR *www.cibmtr.org*, and of cord blood bank networks named Netcord *www.netcord.org* and NMDP *www.nmdp.org*.

Eurocord is an international registry which operates on behalf of the European Blood and Marrow Transplant group (EBMT), which includes European and non-European centers (more than 180 transplant centers in 35 countries), all performing either related or unrelated cord blood transplants. It works in close collaboration with EBMT and Netcord banks to collect clinical data and follow patients transplanted in or outside Europe with Netcord units. The Netcord group was established in 1998 to provide good practice in umbilical cord blood storage, facilitate donor search, improve the quality of the grafts, standardize excellence criteria on an international scale and importantly establish procedures for bank accreditation in collaboration with FACT (Foundation on Accreditation in Cell Therapy). National regulatory agencies and transplant centers are aware of the need for international standards for cord blood collection, processing, testing, banking, selection and release.

All the practical aspects of cord blood banking, such as mother informed consent, collection techniques, labeling and identification, infectious disease and genetic disease testing, HLA typing, methodology of cell processing, cryopreservation, transportation and release have been extensively published. All these aspects are detailed in the last version of the Netcord-FACT Standards (*www.factwebsite.org*).

Currently, there are increasing numbers of international exchanges of cord blood units. For example in France, from January to October 1st 2008, out of an inventory of 6,586 units collected in 3 cord blood banks, 290 units were released: 115 for French patients and 59 for patients abroad. During the same period 175 (65%) were exported abroad from France (Source Biomedicine Agency). As has been described by Querol S *et al.*[13], in this issue of this journal, the optimal number of units is currently estimated at 50,000 for a population of 60 million inhabitants. Of course, this number should increase in relation to the number and origin of ethnic minorities in each country and the current need to select cord blood with the highest number of $CD34^+$ cells and no more than 2 HLA mismatches. As the number of cord blood units is increasing, it appears that it is necessary to improve the quality of the units for cost efficient management of the banks. The optimal number of cord blood units is not really known but should approach 9 per 100,000 inhabitants. Most banks prefer to collect only the largest units of more than 70 mL in order to obtain at least 3×10^7 nucleated cells/kg. The effect of increasing the inventory from 50,000 to 300,000 for finding a matched cord blood with a minimum cell dose of 2.3×10^7/kg increases the chance of finding a donor by 19% for children and 10% for adults.[14] The current recommendations are to choose:

- cord blood units with ≤2 HLA disparities and $>2.5\times10^7$ nucleated cells/kg or $\geq2\times10^5$ $CD34^+$ cells/kg;
- in non-malignant disease where the risk of rejection is higher the dose should be increased and one must avoid units with less than 3.5×10^7 NC/kg and 2 or more HLA incompatibility. If there is no single unit with these characteristics look for 2 units with a combined total dose of $\geq3\times10^7$ NC/kg and if possible not more than 1 HLA difference between the 2 units and the patient.

Other types of cord blood banks have been established such as sibling donor cord blood banking or autologous (or commercial family CB banking) where there is no existing family indication for HSCT. There are 2 types of CBB, public and private according to their economic interest and financial support and 3 types of CBB according to the type of donation and use, unrelated, sibling donor or autologous CBB. Unrelated donor transplantation programs employ public banks as their source of donor cord blood units (CBU). These CBUs are donated on a volunteer basis by women delivering healthy babies at term. Private Banks, which are for-profit entities, store *directed donations* collected by obstetricians from babies born into families who intend to use the cord blood for the baby from whom it came (autologous donation) or for another family member in need of future transplantation therapy.

Future of cord blood transplant

Cord blood transplant needs to meet several new challenges:

- improving the speed of HSCT engraftment and decreasing transplant related mortality. Several possibilities are being investigated: (i) increase the donor pool to decrease the number of HLA mismatches. In the small group of patients who received a 6/6 HLA matched cord blood transplant engraftment is improved, GVH is reduced and survival seems better compared to HLA matched unrelated BMT; (ii) double cord blood transplants: results show better engraftment, more GVH and less relapse compared to patients receiving a single CBT;[15] and (iii) intrabone infusion shows better platelet engraftment and lower incidence of acute GVHD compared to IV infusion (Frassoni F and Rocha V for Eurocord submitted EBMT, 2009). Other methods are currently being investigated such as *ex-vivo* expansion with cytokine cocktails or homing factors.
- use of cord blood for non-hematopoietic transplants. Non-hematopoietic stem cells have been isolated from cord blood and placenta. These cells can be grown and differentiate in various tissues including MSC, bone, cartilage, liver, pancreas, neurones, endothelial cells, muscle, keratinocytes etc… They have an advantage over other sources of stem cells, embryonic stem (ES) cells or induced pluripotent stem cells (iPS), because the supply is unlimited, they can be used in autologous or allogeneic situations, they need minimal manipulation, and they raise no ethical concerns. Future

studies will in the near future test the potential of cord blood cells for the treatment of several diseases including, among other possibilities, diabetes, arteritis, burns, neurological disorders and myocardial infarction.

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Therapy-related myeloid neoplasms

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Therapy-related myeloid neoplasm (t-MN) is the term recently proposed by the World Health Organization to cover the spectrum of malignant disorders previously described as therapy-related myelodysplastic syndrome (t-MDS) or therapy-related acute myeloid leukemia (t-AML). t-MN is a well recognized clinical syndrome occurring as a late complication following cytotoxic therapy.[1-5] The term *therapy-related* leukemia is descriptive and based on a patient's history of exposure to cytotoxic agents. Although a causal relationship is implied, the mechanism remains to be proven. These neoplasms are thought to be the direct consequence of mutational events induced by cytotoxic therapy, or via the selection of a myeloid clone with a mutator phenotype that has a markedly elevated risk for mutational events. Several distinct clinical and cytogenetic subtypes of t-MN are recognized and closely associated with the nature of the preceding treatment. The latency between the primary diagnosis and therapy-related disease ranges between a few months to several years, depending in part on the cumulative dose or dose intensity of the preceding cytotoxic therapy, as well as the exposure to specific agents. The majority of patients have clonal chromosome abnormalities in their bone marrow cells at diagnosis. A spectrum of morphological abnormalities is observed.[4,5] There is a continuum in the percentage of marrow blasts from t-MDS to overt acute leukemia, and rapid progression from the former to the latter. Thus, it is reasonable to consider this as a single clinical syndrome. The clinical course is typically progressive and relatively resistant to conventional therapies used for leukemias arising *de novo*.