An overview of the progress on double umbilical cord blood transplantation

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ABSTRACT

Umbilical cord blood transplantation has been increasingly used over the past years for both malignant and non-malignant hematologic and other diseases as an alternative to mismatched-related or matched-unrelated bone marrow or peripheral blood hematopoietic stem cell transplantation. A disadvantage of cord blood is its low cell content which limits cord blood transplantation to generally low weight recipients, such as children. Various alternatives have been used to overcome this limitation, including co-infusion of two partially HLA-matched cord blood units.

According to Eurocord Registry data, this strategy has been applied in approximately 993 adult patients with hematologic diseases since the first double umbilical cord blood transplantation in 1999. In fact, since 2005, the number of adult patients receiving double umbilical cord blood transplantation has surpassed the number of adults transplanted with single cord blood units. The engraftment rate is comparable for both single and double umbilical cord blood transplantation, although the latter is accompanied by a higher incidence of grade II acute graft-*versus*-host disease and lower leukemia relapse for patients in first complete remission. In the majority of patients undergoing double umbilical cord blood transplantation, transient chimerism, due to the presence of cells from both donor units early post transplant, is replaced by sustained dominance of one unit from which long-term hematopoiesis is derived. Although the biology and the factors that determine unit dominance have not been clarified, the implication of immune-mediated mechanisms has been reported.

Preliminary data have demonstrated the safety of double umbilical cord blood transplantation. Ongoing clinical trials and prolonged follow up of the patients will clarify the immunology and determine the efficacy of this approach. We present here a brief overview of the clinical experience on double umbilical cord blood transplantation and its underlying biology.

Key words: double, umbilical, cord blood, transplant.

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Introduction

Umbilical cord blood transplantation (UCBT), as an alternative to mismatched-related or matched-unrelated bone marrow or peripheral blood stem cell transplantation, has been increasingly used over the past years for both malignant and non-malignant hematologic and other diseases. Since the first UCBT in 1988 for Fanconi's anemia, more than 20,000 have been performed, mainly in children.[1-4] The major factor which compromises the application of unrelated UCBT in adults is the relatively low number of progenitor cells present in umbilical cord blood and HLA disparity.[3,5] Increasing cell dose has been reported to improve engraftment, especially in adults, and partially overcome the influence of HLA disparities (up to 2).[1,2,3,6,7,8,9] On the other hand, HLA compatibility is crucial for the survival of patients undergoing UCBT for non-malignant diseases, but there is still controversy over HLA disparity being associated with a decrease in relapse in the case of malignancies due to the possible increased alloreactivity to both the host and tumor cells, which is known as graft-*versus*-leukemia (GvL) effect.[6,9]

To overcome the low cell content of single UCB units, various alternatives have been used. Multidonor UCBTs of up to 12 units have shown that crossed immunological reaction between the units does not occur.[10-16] Equally encouraging were the results of a study involving co-infusion of a related umbilical cord blood graft and a haploidentical or third-party donor peripheral blood graft (dual transplant) in patients with high-risk hematologic malignancies.[17,18] However, with this approach, the potential development of severe acute graft-*versus*-host disease (GvHD) that could be triggered by the haploidentical $CD34^+$ cells is a major concern. Within the context of dual transplant, the co-infusion of unrelated umbilical cord blood with mobilized hematopoietic stem cells (HSCs), whether or not these are T-cell depleted, from third-party donors has been used in clinical practice.[19] Compared to single UCBT, this approach is accompanied by shorter periods of neutropenia due to early engraftment of the mobilized hematopoietic stem cells, and therefore a lower incidence of graft-*versus*-host disease. Studies involving *in vitro* expansion of a fraction of the umbilical cord blood with cytokines and subsequent co-infusion of the expanded and non-expanded

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units reported engraftment, but there was no difference in median time to neutrophil recovery to that observed with non-expanded umbilical cord blood.[20] The infusion of a $CD34^+$ selected and *ex vivo* expanded unit *via* the Notch signaling pathway unit alongside an unmanipulated umbilical cord blood unit has also been reported with improved time to neutrophil recovery compared to the infusion of 2 unmanipulated units.[21] The simultaneous transplantation of 2 partially HLA-matched umbilical cord blood units (double umbilical cord blood transplantation, dUCBT) has also been used to overcome cell dose limitations. We present here a brief literature overview of the clinical experience from dUCBT and its underlying biology.

Double umbilical cord blood transplantation

The first dUCBT was performed in Europe in 1999 on 2 adults with acute lymphoid and chronic myeologenous leukemia.[22] Both patients had signs of donor engraftment but died three months post transplant; one from relapse and the other from hemorrhage (Eurocord, unpublished data, 2010).[22] In 2001, the first case of dual donor chimerism was reported by Barker *et al.* following transplantation of 2 partially HLA-matched unrelated umbilical cord blood donors.[13] In 2005, the same group published the safety and feasibility of dUCBT in 21 adults undergoing myeloablation for malignant diseases, and a few years earlier (2003) used dUCBT as a strategy for patients with no adequately sized units in a study regarding the outcome of UCBT with reduced intensity conditioning (RIC) regimens.[23,24] The results were encouraging, as the percentage of engraftment was increased, whereas relapse and severe acute graft-*versus*-host disease were decreased. Since then, dUCBT has been performed mainly in patients with hematologic malignancies undergoing both non-myeloablative and myeloablative conditioning (Table 1). Amongst these, 84 dUCBTs were carried out on children (n=45) and adults (n=39) with non-malignant diseases, mainly with bone marrow failure syndromes.[22] According to Eurocord reports, since 2005, the number of adult patients receiving dUCBT has surpassed the number of adults transplanted with single units.[22]

Conditioning regimens

Conditioning regimens of variable intensity have been used to reduce the complications associated with dUCBT and improve its outcome. In the context of myeloablation, high-dose total body irradiation (TBI) either alone or in combination with various chemotherapeutic agents (mainly cyclophosphamide and fludarabine) has been used.[25,25,26,30,31,32,34] Reduced intensity conditioning regimens, along with immunosuppressants, have also been successfully used to minimize the undesirable toxic effects of myeloablation while producing sufficient immunosuppression to promote long-term engraftment.[24,27-33] Majhail *et al.* recommended reduced intensity conditioning in patients over 55 years of age undergoing dUCBT.[35] Such patients were previously excluded from transplant on the basis of age and lack of a suitable matched related or unrelated donor. Recently, Eurocord published a study involving single and double UCBT on 104 patients with lymphoid malignancies using both conditioning regimens and concluded that a better outcome was achieved in chemosensitive patients receiving low-dose total body irradiation and high cell dose.[31,32]

Graft characteristics

The current recommendations for UCBT are: i) cord blood units with no more than 2 HLA disparities and 3×10^7 or more nucleated cells/kg or 2×10^5 $CD34^+$ or more cells/kg. For units that are HLA-matched (6/6 units) and mismatched (4-5/6) the minimum recommended cell dose is 3×10^7 or more and 4×10^7 or more nucleated cells/kg, respectively; ii) in cases in which the risk of rejection is higher, such as in non-malignant diseases, the cell dose should be higher than 3.5×10^7 or more nucleated cells/kg and HLA disparity between the units and the patient of not more than 1; iii) if no single unit meeting these requirements is available, it is recommended to use 2 units with a combined cell dose of 3×10^7 or more nucleated cells/kg and, if possible, no more than 2 HLA disparities between the units and the recipient or between the units.[22,36,37,38]

The role of HLA matching, and the interaction between cell dose and the extent of HLA disparity have not been fully clarified.[9] Bearing in mind that HLA disparity is associated with survival and hematopoietic recovery in UCBT, higher cell dose can abrogate the effect of HLA mismatching, as long as there are no more than 2 HLA incompatibilities between the patient and each unit.[9,33,39,40] There are few data on the effect of locus-specific HLA matching in UCBT beyond HLA-A, B and DR; usually allele-level typing for locus HLA-DRB1 and antigen-level typing for HLA-A and B.

In dUCBT, the units are administered intravenously either sequentially (within 30 min) or within 6 h apart, after confirming that the first unit has been successfully infused.[41] Intrabonal administration has also been used and found to be well-tolerated, safe and comparable to the conventional intravenous administration of the 2 units.[42,43]

Chimerism

More than 85% of patients undergoing dUCBT demonstrate rapid skewing of donor engraftment and a single unit emerges as the 'winner' to sustain long-term hematopoiesis (i.e. at least 90% marrow reconstitution of recipient by donor).[23-34,38,44] The time frame for determining dominance has not yet been clarified.[45] Usually, by day 21 post transplant single unit dominance can be detected in over 80% of patients, although dominance as soon as 14 days post transplant has also been reported.[38,46] There can also be mixed chimerism (i.e. presence of both donor units) at varying ratios, especially in patients undergoing reduced intensity conditioning regimens.[47] Algorithms are available that can provide approximations of the chimerism pattern following dUCBT.[48] Dominance reversion has rarely been reported, except for a 15-year old boy treated with dUCBT for acute lymphoblastic leukemia in whom long-term mixed donor chimerism and dominance reversion (on day 253 post transplant) were observed during follow up for more than 16 months (479 days post transplant).[49] Furthermore, in a phase I dUCBT clinical trial involving patients with hematologic malignancies undergoing a reduced intensity conditioning regimen, a patient with 95% single donor chimerism on day 65 post transplant was reported to lose single unit dominance in favor of mixed donor chimerism over time.[41] The patient died approximately 200 days post transplant from a lymphoproliferative disorder.

The parameters that influence umbilical cord blood predominance in dUCBT have not been clarified. There is no

correlation between dominance and the number of nucleated cells, CD34+, CD3+, degree of HLA/sex mismatch, high resolution HLA matching, ABO group, viability, order and route of infusion.[38,44] Preliminary evidence, however, suggests that cord blood units with low CD34+ cell viability (<75%) have low probability of engraftment upon co-infusion with a unit of high CD34+ cell viability (>75%).[50] Whether dominance is influenced by intrinsic features of the units, host-*versus*-graft and/or graft-*versus*-graft interactions must still be determined. It is possible

Table 1. Double umbilical cord blood transplantation studies.

Group	Kai[25]	Barker[23]	Yoo[26]	Ballen[27]	*Brunstein[28]	Ruggeri[29]	Bradstock[30]	*MacMillan[31]	*Rodrigues[32]	*Rocha[33]	*Verneris[34]
Patients (n.)	11	23	12	21	93	14	11	185	26	59	93
Median patient age, range (years)	33 (19-52)	24 (13-53)	13.3 (6.2-16.9)	49 (18-65)	51 (17-69)	16 (6-31)	27 (17-58)	45 (10-69)	41 (16-65)	47 (18-69)	24 (9-57)
Median patient weight, range (kg)	68 (48-84)	73 (48-120)	NS	78 (58-111)	76 (50-134)	45 (17-72)	69 (44-113)	78 (33-149)	68 (39-130)	NS	69 (32-149)
Median follow up of surviving patients, range (months)	16	10 (3.5-30)	18	18 (11-30)	19 (4.8-51)	23 (10-27)	17-33	26.4 (12-63.6)	18 (3-74)	18 (2-56)	32.4 (6-84)
TNC dose, range (x10^7/kgr recipient)	3.88 (2.83-4.79)	3.5 (1.1-6.3)	3.13 (0.72-6.2)	4 (2.9-5.1)	3.7 (1.5-6.8)	4.8 (0.9-8.40)	4.7 (2.0-7.8)	3.6 (1.1-8.0)	3.02 (1.20-7.90)	3.6	3.6 (1.1-6.5)
Total CD34+ cells, range (x10^5/kgr recipient)	1.06 (0.62-2.6)	4.9 (1.2-14.5)	NS	1.9 (0.6-9.7)	4.9 (0.7-16.6)	2.9 (0.5-7.46)	1.3 (1.0-1.8)	4.7 (0.7-1.7)	0.91 (0.14-5.15)	1.6	4.5 (0.9-14.5)
Patients receiving at least 1 unit 2 HLA-mismatched to the recipient (%)	NS (at least)	43	NS	76	79	64	73	57	73	60	65
Conditioning (n. of patients)	MAC	MAC	MAC	RIC	RIC	RIC	MAC (10)/ RIC (1)	MAC (78)/ RIC (107)	MAC/RIC	RIC	MAC
Neutrophil engraftment, median day (range)**	21 (16-26)	23 (15-41)	23 (15-34)	20 (15-34)	12 (0-32)	28 (14-42)	32 (18-53)	NS	17	20	25 (8-41)
Cumulative neutrophil recovery (%)	82	100	100	NS	92	57	NS	NS	92	80	86
Platelet engraftment, median day (range)**	53 (32-98)	NS	47 (24-81)	41 (21-55)	49 (0-134)	105 (36-180)	91 (56-381)	NS	NS	NS	NS
Cumulative platelet recovery (%)	82	71	87	NS	65	57	NS	NS	NS	NS	62
aGvHD II-IV (%)	NS	65	66.7	40	62	71	36	58[MAC: 53/ RIC: 62]	24	37	48
aGvHD III-IV (%)	NS	13	16.7	NS	22	NS	NS	19	8	NS	25
cGvHD (%)	36	23	25	25	23	43	-	17	18	39	18
TRM (%) [months post transplant]	NS	22 [6]	16.7 [18]	19% [6]	19 [6]/26 [36]	NS	45 [1-2]	24 [12];	NRM 31 [12]	NRM 18 [18]	29 [12]
DFS (%) [months post transplant]	NS	CML and CR acute leukemia patients: 57 [12]/ patients with leukemia in relapse or recurrent MDS: 25 [12]	71 [18]	67 [12]/ 55 [24]	39 [36]	Predicted 50% [24]	36 [17-33]	NS	57 [12]	Predicted 51 [18]; 70% and 42% for UCB units >4 and ≤4/6 respectively HLA-matched to patient	LFS: 58 [12]/ 51 [60]
Chimerism pattern in surviving patients [days post transplant]	SUD [28]	SUD [100]	SUD (8 patients)/ MUC (1 patient) [365]	SUD in 76% of patients [90]	SUD [365]	SUD [100]	Long-term SUD	Long-term SUD	SUD (16 patients)/ MUC(1 patient)	NS	Long-term SUD

*NS: not stated; TNC: total nucleated cells; MAC: myeloablative chemotherapy; RIC: reduced intensity conditioning; aGvHD: acute GvHD; cGvHD: chronic GvHD; TRM: treatment-related mortality; NRM: non-relapse related mortality; DFS: disease-free survival; CML: chronic myelogenous leukemia; CR: complete remission; MDS: myelodysplasia; LFS: leukemia-free survival; SUD: single unit dominance; MUC: mixed unit chimerism. * The studies of Brunstein, MacMillan, Rodrigues, Rocha and Verneris included single and double umbilical cord blood transplantations (UCBTs) in parallel, but the table demonstrates the results of dUCBTs only. ** Times of neutrophil and platelet engraftment were defined as the first of three consecutive days measured from the date of transplantation of absolute neutrophil and platelet counts at least $0.5x10^9$ neutrophils/L and $20x10^9$ platelets/L respectively. For platelet engraftment the patients were also without platelet transfusion support for seven days.*

that the non-engrafted unit might facilitate/activate the engraftment of the sustained unit. On the other hand, the use of 2 units might simply increase the chances of infusing a unit with engrafting potential.[38] Predicting the 'winning' unit might, therefore, be impossible (e.g. "atmospheric noise" theory).[51] Interestingly, patients with mixed chimerism following reduced intensity conditioning dUCBT tend to be more prone to chronic graft-*versus*-host disease at one year post transplantation, which suggests that the conditioning regimen might interfere with the chimerism pattern.[27] This observation could also indicate graft-*versus*-graft interactions and is consistent with recent *in vivo* evidence demonstrating that naive $CD8^+$ T cells in one unit expanded and differentiated into IFN-γ secreting effector T cells that specifically recognized the non-engrafting unit and mediated its rejection.[46] These cells were only detected transiently in the peripheral blood of dUCBT recipients with single unit dominance and are, therefore, not likely to be the sole cause of rejection. Further evidence in favor of a T-cell mediated graft-*versus*-graft effect is provided by studies on both mice and patients. According to these, the infusion of 2 $CD34^+$ units resulted in mixed chimerism, whereas the addition of the corresponding mononuclear cells or $CD34^-$ cells restored single unit dominance.[52,53] Bearing in mind that the 2 units are mismatched at one or more HLA alleles, the alloreactive response could be specific for major or minor allogeneic determinants, such as minor H antigens that are shared between umbilical cord blood units. This hypothesis can explain the enhanced graft-*versus*-leukemia effect associated with dUCBT if the major or minor H antigens expressed on hematopoietic stem cells of the non-engraft-

Table 2. Comparison of selected parallel single *versus* double umbilical cord blood transplantation studies.

Group	Brunstein[28]		MacMillan[31]		Rodrigues[32]		Verneris[34]	
	sUCBT	dUCBT	sUCBT	dUCBT	sUCBT	dUCBT	sUCBT	dUCBT
Patients (n.)	17	93	80	185	78	26	84	93
Median patient age, range (years)	51 (17-69)		23 (10-65)	45 (10-69)	41 (16-65)		8 (0.5-5.2)	24 (9-57)
Median patient weight, range (kg)	76 (50-134)		63 (30-107)	78 (33-149)	68 (39-130)		32 (9-108)	69 (32-149)
Median follow up of surviving patients, range (months)	19 (4.8-51)		58 (24-110)	26.4 (12-63.6)	18 (3-74)		68.4 (18-144)	32.4 (6-84)
TNC dose, range ($x10^7$/kgr recipient)	3.3 (1.1-5.3)	3.7 (1.5-6.8)	2.4 (0.9-14.0)	3.6 (1.1-8.0)	2.41 (0.88-10.20)	3.02 (1.20-7.90)	3.3 (0.9-14)	3.6 (1.1-6.5)
Total $CD34^+$ cells, range ($x10^5$/ kgr recipient)	3.8 (1.2-18.8)	4.9 (0.7-16.6)	2.8 (0.5-1.9)	4.7 (0.7-1.7)	1.07 (0.06-4.30)	0.91 (0.14-5.15)	3.5 (0.4-34.8)	4.5 (0.9-14.5)
Patients receiving at least 1 unit 2 HLA-mismatched to the recipient (%)	65	79	56	57	60	73	42	65
Conditioning (n. of patients)	RIC		MAC (61 patients)/ RIC (19 patients)	MAC (78 patients)/RIC (107 patients)	MAC/RIC		MAC	
Neutrophil engraftment, median day (range)*	12 (0-32)		NS	NS	17		22 (9-38)	25 (8-41)
Cumulative neutrophil recovery (%)	92		NS	NS	81	92	70	62
aGvHD II-IV (%)	41	62	39 [MAC:38/RIC:42]	58 [MAC:53/RIC: 62]	22**	32**	29	48
aGvHD III-IV (%)	22		18	19	8		12	25
cGvHD (%)	23		18	17	18		10	18
TRM [years post transplant (%)	26 [3]		39 [1]	24 [1]	NRM: 26 [1]	NRM:31 [1]	26 [1]	29 [1]
DFS (%) [years post transplant]	24 [3]	39 [3]	NS	NS	PFS: 35 [1]	PFS: 57 [1]	LFS: 55 [5] [1]/40	LFS: 58 [1]/51 [5]
Overall survival (%) [years post transplant]	40 [3]	45 [3]	NS	NS	42 [1]	65 [1]	47 [5]	
Relapse (%) [years post transplant]	41 [3]	30 [3]	NS	NS	38 [1]	13 [1]	Overall 34 [5]/ CR1-2 patients: 31	Overall 19 [5]/ CR1-2 patients:16

*sUCBT: single umbilical cord blood transplantation; dUCBT: double umbilical cord blood transplantation; TNC: total nucleated cells; MAC: myeloablative chemotherapy; RIC: reduced intensity conditioning; aGvHD: acute GvHD; cGvHD: chronic GvHD; TRM: treatment-related mortality; NRM: non-relapse related mortality; DFS: disease-free survival; NS: not stated; PFS: progression-free survival; LFS: leukemia-free survival; CR: complete remission. * Time of neutrophil engraftment was defined as the first of three consecutive days measured from the date of transplantation of absolute neutrophil count at least $0.5x10^9$ neutrophils/L. ** The grade of aGvHD was not specified.*

ing unit are common with those expressed on leukemic stem cells, although it is not clear whether HLA disparity also contributes. The identification of the antigens expressed on hematopoietic stem cells to which T cells of the dominant unit are responsive is ongoing. On the other hand, mixed chimerism can be explained on the basis of $CD4^+$ T cells that develop *in utero* and promote tolerance to non-inherited maternal alloantigens that are by chance shared by the other umbilical cord blood unit.[46,54] The contribution of other immune-related effector mechanisms, such as killer immunoglobulin-like receptor-ligand differences and NK-cell activation, are under study.[55,56]

Co-transplantation of mesenchymal stem cells (MSCs) from various sources (bone marrow, placenta) has been shown to marginally improve the engraftment of hematopoietic stem cells in clinical studies.[31,57-59] In murine models, dUCBT combined with co-transplantation of placental or bone marrow mesenchymal stem cells resulted in improved engraftment and reduced dominance of a single unit in favor of mixed chimerism.[57,61] Mesenchymal stem cells secrete a broad range of immune-related bioactive molecules, such as cytokines, growth factors and chemokines that have both autocrine and paracrine activities, such as turning off T-cell surveillance (i.e. trophic activity).[60] Also, culture-expanded mesenchymal stem cells do not express MHC class II surface markers and co-stimulatory molecules and cannot, therefore, function as antigen-presenting cells; they are invisible to the host's immune system.[62-64] They could, therefore, interfere with immunosurveillance and modify graft-*versus*-graft interactions so that, although one donor unit still dominates, the degree of dominance is reduced.[57,61]

It has been reported that when the units are infused intravenously within 3.5-4.5 h apart, the predominant unit is usually the first to be infused.[27,41] It has also been demonstrated that non-lineage hematopoietic stem cells can home to the endosteal region of the niche in under five hours.[65] Even a short interval in infusion may, therefore, confer advantage to the unit infused first, making it more likely to fill the niche space, which is limited to maintain tight long-term regulation between proliferation and quiescence of the resident stem cells.[27,41,66] The combination of intravenous and intrabone administration was not shown to confer selective advantage in predominance of engraftment.[43]

These studies could provide evidence in favor of both graft-*versus*-graft interactions between the 2 units. Gutman *et al.* observed a great variation *in vitro* regarding the proliferative potential of $CD34^+$ cells from umbilical cord blood.[46] It is, therefore, likely that intrinsic factors concerning the stem cells which are not yet fully understood, such as homing to the niche, as well as prior therapy, intensity of conditioning regime, trophic effects and host factors, are all likely to contribute to the pattern of chimerism in dUCBT.

Survival (GvHD, GvL, infectious complications)

dUCBT is accompanied by increased acute graft-*versus*-host disease which varies according to the conditioning regimen and immunoprophylaxis, while the incidence of chronic graft-*versus*-host disease is roughly similar in all studies (Table 1). There is also a trend for reduced intensity conditioning dUCBT patients with mixed chimerism to be more prone to chronic graft-*versus*-host disease at one year post transplantation.[32] Furthermore, there is increasing evidence to suggest improved leukemia-free survival for leukemia patients undergoing dUCBT in remission at the time of transplant.[28,32,33,34,38,67]

HLA matching is important for engraftment and to minimize risk of relapse, especially for leukemia patients.[9,34] However, high resolution HLA matching does not affect either overall survival nor disease-free survival, nor can it predict dominance in dUCBT.[40] The effect of HLA disparities, especially of the engrafting unit to the recipient with respect to acute graft-*versus*-host disease onset has not been fully clarified. Following dUCBT under reduced intensity conditioning, Delaney *et al.* reported that close HLA-DR matching was associated with a trend for lower risk of incidence of acute graft-*versus*-host disease, and HLA-B matching was associated with faster neutrophil and platelet engraftment.[40]

Reporting on infectious complications following dUCBT is limited, due to small cohort size and availability of only short-term patient follow-up data. In general, the prolonged neutropenia and monocytopenia that accompany UCBT increase susceptibility to bacterial, fungal and opportunistic viral infections. Approximately 30% of the deaths following dUCBT can be attributed to infections, amongst which a relatively higher incidence of BK-virus, Epstein Barr virus, adenovirus and HHV6 have been reported.[68] In fact, HHV6 virus was detected almost systematically in blood samples from dUCBT patients, with no clinical manifestations.[68] Prophylactic citomegalovirus treatment in dUCBT patients is generally not considered necessary.[69] It has been suggested that as long as a sufficient dose of total nucleated cells is infused and sustained neutrophil recovery is established, infection-related mortality is not excessively high.[68] Other complications are rarely reported.[70] The main cause of death is the progression and relapse of the original disease. Myelogenous malignancies of donor origin have also been reported to occur, suggesting that careful donor selection is necessary for all patients with suspected relapse.[71]

Clinical experience with dUCBT

From 1999 to March 2010, 1,152 dUCBTs, combined with conditioning of various intensities, have been performed in patients with hematologic malignancies who could not find suitable unrelated donors.[22] The combination of reduced intensity conditioning and dUCBT in particular have extended the availability of transplantation to older or heavily treated patients who have an increased risk of treatment-related mortality.[24,27-29,31-33,35] More than 300 patients, both children and adults who had no alternative treatment option for leukemia, have also received dUCBT at the University of Minnesota since 2001.[27]

The preliminary encouraging data on the safety and feasibility of dUCBT have resulted in the launch of randomized clinical trials, investigating either the outcome of dUCBT or comparing single *versus* double UCBT in children and adults with hematologic malignancies. However, little published evidence is currently available on the long-term immune reconstitution and clinical benefit of dUCBT. Although similar neutrophil engraftment kinetics have been observed in a small study involving adults undergoing dUCBT and children undergoing single UCBT, an overview of studies on relatively large cohorts of patients comparing the outcome of single *versus* double UCBT in parallel suggests that dUCBT is accompanied by: i) a higher incidence of acute graft-*versus*-host disease grade II, though

not higher treatment-related mortality or chronic graft-*versus*-host disease; ii) lower leukemia relapse for patients with good disease status (complete remission 1-2), indicating a potentially higher graft-*versus*-leukemia effect (Table 2).[28,31,32,34,72,73] MacMillan *et al.* also reported that acute graft-*versus*-host disease occurred sooner in dUCBT recipients (median day 28 *vs.* 36).[31] In the same study, treatment-related mortality after the onset of acute graft-*versus*-host disease was significantly lower in dUCBT patients (20% *vs.* 39% one year post transplant).[31]

Future

Umbilical cord blood is being increasingly used as a source of hematopoietic stem cells in allogeneic transplantations for patients who do not have an available HLA-matched donor. To overcome the low cell number, the co-transplantation of 2 umbilical cord blood units (dUCBT) is a promising strategy with comparable engraftment rate and potentially higher graft-*versus*-leukemia effect compared to single UCBT.

The biology underlying dUCBT and the factors that determine unit dominance are not yet fully understood. The implication of immune-mediated mechanisms raises the question that stem cells HLA-matched to the recipient unit, even of low cell dose, could be combined with a higher cell dose unit to improve the outcome in dUCBT. This could also be of significance in the context of leukemia; if the dominant unit can be predicted prior to transplantation, a non-engrafting unit sharing host antigens not present on the engrafting unit can be selected to promote the graft-*versus*-leukemia effect.

A reasonable limitation of dUCBT is the cost of 2 umbilical cord blood units, especially from unrelated donors, and the costs of hospitalization due to the low engraftment rate.[22,74] Various approaches to improve engraftment and enhance immune reconstitution after dUCBT are being evaluated, such as infusions of cells (NK cells and cytotoxic T lymphocytes) with antiviral and antileukemic specificities, co-culture or co-transplantation with mesenchymal stem cells, as well as *ex vivo* expansion with cytokines and/or homing factors.[75] The ongoing clinical trials will clarify the therapeutic benefit of dUCBT in a variety of malignant and non-malignant diseases in children and adults without an HLA-matched sibling donor.

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