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REVIEW

Cellular engineering and therapy in combination with cord blood allografting in pediatric recipients

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Cord blood (CB) transplantation is an alternate source of human hematopoietic progenitor cells for allogeneic stem cell transplantation in children and adolescents with both malignant and nonmalignant diseases. Current limitations included delay in hematopoietic reconstitution, increased incidence of primary graft failure and slow cellular immunoreconstitution. These limitations lead to a significant increase in primary graft failure, infectious complications and increased transplant-related mortality. There is a number of experimental approaches currently under investigation including cellular engineering to circumvent these limitations. In this review, we summarize the recent findings of utilizing *ex vivo* CB expansion with Notch1 ligand Delta 1, mesenchymal progenitor cells, the use of human placenta-derived stem cells and CB-derived natural killer cells. Early and preliminary results suggest some of these experimental cellular strategies may in part ameliorate the incidence of primary graft failure, delays in hematopoietic reconstitution and/or slowness in cellular immune reconstitution following unrelated CB transplantation.

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INTRODUCTION

We and others have reported the success of unrelated cord blood transplantation (UCBT) in children with malignant and nonmalignant diseases.[1–7] There is a number of advantages of unrelated cord blood (UCB) versus other unrelated stem cell sources, such as rapid availability, multiethnic representation, immaturity of T-cell immunity, decreased severe (Grade III/IV) acute GVHD and reduced incidence of chronic GVHD.[1,2,5] However, there are some disadvantages of utilizing UCB including slower and delayed hematopoietic recovery and immune reconstitution, limited cell dose and higher incidence of graft failure (Table 1).[1,2,5] The probability of neutrophil engraftment following UCBT is significantly associated with the pre-thaw total nucleated cell dose (TNC)/kg dose infused and leukemia-free survival following UCBT is significantly associated with both TNC/kg and HLA matching.[3,8] The TNC count in UCB is highly correlated with the pre-thaw $CD34^+$ cell content and the $CD34^+$ cell/kg cell dose following UCBT is significantly associated with overall survival (OS) as we and others have reported before.[9,10]

The use of reduced toxicity conditioning before UCBT is associated with similar rates of neutrophil engraftment and immune reconstitution but a higher risk of primary graft failure. Primary graft failure following UCBT, however, is associated with a significant increase in transplant-related mortality.[11,12] Although initial studies of double UCBT in adults appeared to be encouraging,[13,14] a recent prospective randomized trial of single versus double UCBT in children with hematological malignancies demonstrated no improved overall survival and a significant increase in severe acute GVHD and chronic GVHD in double cord transplant recipients.[15] New approaches are needed to accelerate the rapidity of neutrophil engraftment, and cellular immune reconstitution, reduce primary graft failure, decrease transplant-related mortality and subsequently enhance OS following UCBT. This report summarizes a few new therapeutic approaches including *ex vivo* expansion, using engineered cord blood (CB) $CD34^+$ cells expressing the Notch ligand Delta 1, co-culture expansion of mesenchymal progenitor cells (MPC) with CB progenitor cells, the addition of third party human placenta-derived stem cells (HPDSCs) with single or double UCBT and the expansion and utilization of CB-derived natural killer (NK) cells.

EX VIVO EXPANSION OF CB HEMATOPOIETIC AND PROGENITOR CELLS

The *ex vivo* expansion of CB-derived hematopoietic stem and progenitor cells (HSPC) as a strategy to increase the $CD34^+$ cell/kg dose and enhance the kinetics of engraftment is the furthest along clinically and under investigation by a number of investigators (Table 2). Extremely promising results have been reported by these various investigators, including our own work (CD) using an engineered form of the Notch ligand Delta 1 for the *ex vivo* generation of increased numbers of CB $CD34^+$ HSPC with the goal of reducing the time to engraftment (Figure 1). Preliminary results, reported by Delaney *et al.* demonstrated both safety and clinical feasibility of this approach as well as a significant decrease in the time to neutrophil recovery.[16] Updated data (unpublished) from this ongoing study now with 22 patients

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show a median time to neutrophil recovery (ANC ⩾ 500) of 11 days regardless of UCB source following HLA-matched CB units compared with 25 days in a concurrent institutional cohort of patients ($N = 40$) treated with the same conditioning regimen and a double CB graft. In the partially HLA-matched CB units, the median time to ANC recovery was 19 days (Table 2). Of note, the expanded cell graft in this study contributed almost exclusively to initial myeloid engraftment observed at 1 week, demonstrating an enhanced capacity of the expanded cells to provide rapid myeloid recovery. Furthermore, all but two evaluable subjects engrafted before day 21, independent of whether the expanded cell graft persisted *in vivo*. Of note, the unit that was expanded *ex vivo* underwent positive selection for $CD34^+$ cells to initiate in culture, and the negative fraction from this unit was not infused at the time of transplant. This approach of Notch ligand Delta 1 expression is designed to expand only one UCB unit and not the other and thereby results in the expansion of hematopoietic progenitor cells but lymphoid progenitor cells would not be expected to enhance lymphoid reconstitution. Other approaches using Notch ligand Delta 4 are being investigated to expand lymphoid progenitor cells following UCBT.[17]

As shown in Table 2, there are now quite a few clinical approaches utilizing different methods for the *ex vivo* expansion of CB-derived progenitors, ranging from mesenchymal stem cell co-cultures to strategies that target molecular pathways involved in stem cell self-renewal and cell fate. The methods in Table 2 are all methods that are under clinical investigation and have been reported as least in preliminary form in abstracts or publications.[16,18,19] Each of these studies involved limited numbers of patients, but all were conducted in patients undergoing cord blood transplantation for hematologic malignancies. All methods resulted in an increased absolute number of $CD34^+$

Table 1. Advantages and disadvantages of cord blood and cord blood transplantation

Advantages of cord blood and cord blood transplantation
Ease and safe procurement
Rapid availability
Decreased viral transmission
Multi-ethnic representation
Enriched HPC
Immaturity of T-cell immunity
Decreased severe AGVHD
Reduced chronic GVHD
Disadvantages of cord blood and cord blood transplantation
Decreased supply
Expensive to develop
Limited cell dose
Genetic/infectious transmission
Higher incidence of graft failure
Delay in hematopoietic recovery
Prolonged immune reconstitution
Lack of available cells for adoptive cellular therapy
Increased infectious morbidity

Abbreviation: AGVHD = acute GVHD; HPC=hematopoietic progenitor cells.

Table 2. Comparison of *ex vivo* cord blood expansion studies

Group	*Manipulation*	N	*$CD34^+$ cell fold expansion*	*$CD34^+$ cell/kg median ($x\ 10^6$)*	*Days to ANC 500*
Delaney *et al.*[16] FHCRC	Notch—fresh 16-day culture	23	178 (14–481)	8.3 (0.9–49)	11 (6–41)
Delaney *et al.*[19] FHCRC	Notch—TPD cryopreserved	15	176 (32–748)	6 (3.1–11.6)	19 (9–31)
Shpall and colleagues[18] Mesoblast	Co-culture MSC 14 days	24	30.1 (0–138)	0.95 (1.60–9.34)	15 (9–42)
Wagner *et al.*[56] Novartis	SR1—fresh+T 15 days	9	248 (66–446)	11 (1.4–49)	16 (6–43)
Horwitz *et al.*[53] Gamida cell	Nicord—fresh+T 21-day culture	11	72 (16–186)	3.5 (0.9–18.3)	13 (7 26)

Abbreviations: FHCRC = Fred Hutchinson Cancer Research Center; MSC = mesenchymal stem cell; SR1 = StemRegenin 1; TPD = third party donor.

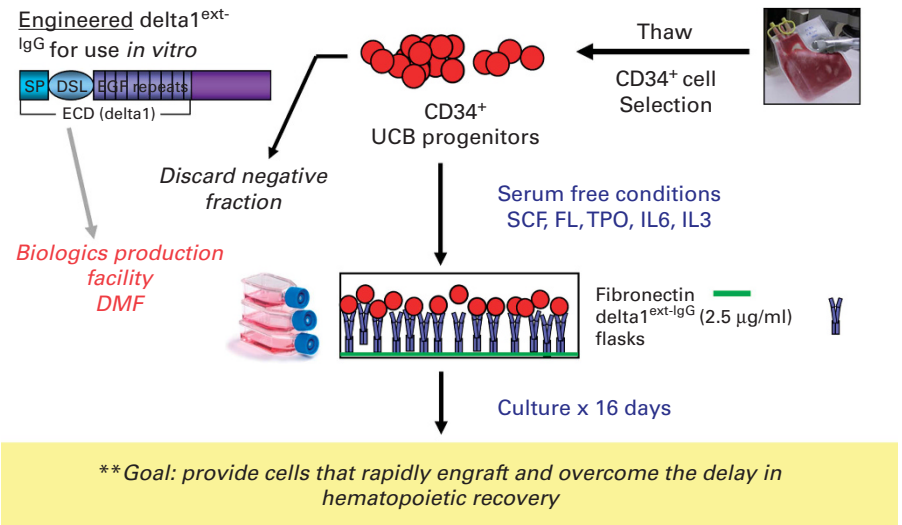
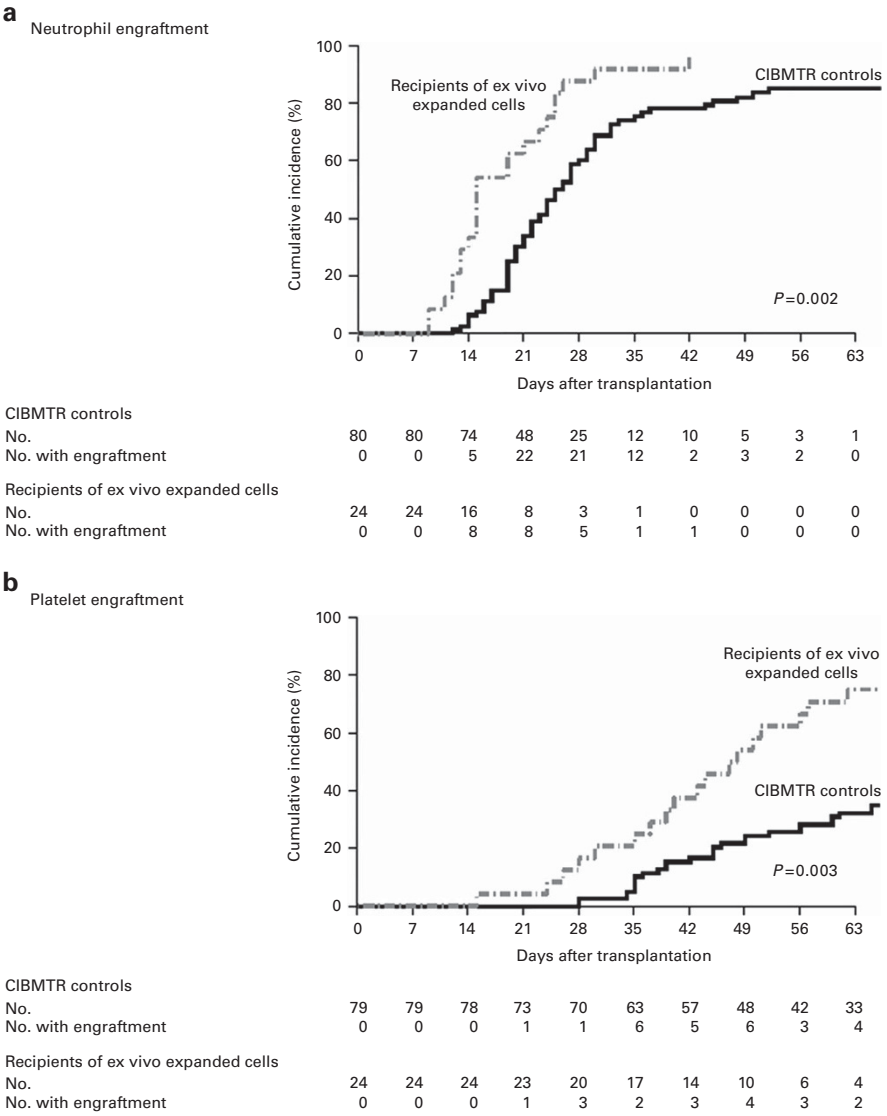


Figure 1. Engineered notch ligands: Translation from bench to bedside. In this cartoon, one UCB is selected for $CD34^+$ cells, the negative fraction is discarded and the $CD34^+$ cell enriched fraction is expanded by Notch ligand Delta 1 in combination serum-free stem cell factor (SCF), FLT-3 ligand (FL), TPO, interleukin 6 (IL6) and interleukin 3 (IL3) in fibronection-coated flasks and cultured *ex vivo* in a biological GMP product facility for ~16 days and then infused with another CBU.



CIBMTR controls										
No.	80	80	74	48	25	12	10	5	3	1
No. with engraftment	0	0	5	22	21	12	2	3	2	0
Recipients of ex vivo expanded cells										
No.	24	24	16	8	3	1	0	0	0	0
No. with engraftment	0	0	8	8	5	1	1	0	0	0

CIBMTR controls										
No.	79	79	78	73	70	63	57	48	42	33
No. with engraftment	0	0	0	1	1	6	5	6	3	4
Recipients of ex vivo expanded cells										
No.	24	24	24	23	20	17	14	10	6	4
No. with engraftment	0	0	0	1	3	2	3	4	3	2

Figure 2. Cumulative incidences of neutrophil engraftment and platelet engraftment.[18] A total of 24 patients who received 2 units of cord blood, 1 of which contained cord blood that was expanded *ex vivo* in co-cultures with STRO-3+ mesenchymal cells, were compared with 80 control patients who received 2 units of unmanipulated cord blood and whose data were reported to the Center for International Blood and Marrow Transplant Research (CIBMTR). Controls were matched according to age, diagnosis, intensity of the preparative regimen and prophylaxis against GVHD. (**a**) The cumulative incidence of neutrophil recovery. At 26 days, the cumulative incidence was 88% among recipients of expanded cord blood and 53% among CIBMTR controls ($P < 0.001$). (**b**) The cumulative incidence of platelet recovery. At 60 days, the cumulative incidence was 71% among recipients of expanded cord blood and 31% among CIBMTR controls ($P < 0.001$). Data on platelet engraftment were not available for one CIBMTR control. *Ex vivo* expansion led to more rapid neutrophil and platelet engraftment and to a higher proportion of patients with engraftment of both cell types. Used from de Lima *et al*.[18]

cells for infusion, to varying degrees, and all reduced the time to neutrophil recovery. However, the clinical efficacy and feasibility of these approaches remain ill-defined and will require larger studies. It is likely that a combination of these approaches will be required to reach the full potential of CB graft engineering.

CB EXPANSION WITH MPC

Another recent approach has been the use of third party MPC to enhance engraftment following second party UCBT.[18] de Lima *et al*. demonstrated enhancement of neutrophil and platelet engraftment following transplants with UCB co-cultured *ex vivo* with MPC (Figure 2).[18] The co-culture of CB with MPC significantly increases both the CB TNC and CD34 counts. Both the increase in TNC/kg and CD34/kg of the final CB unit were significantly associated with an accelerated time to neutrophil recovery (Figure 3).[18] Shpall *et al*. is currently investigating in a randomized study in children and adults with selected hematological malignancy the time to neutrophil recovery following two unmanipulated CB units versus one unmanipulated CB unit plus one partially matched CB unit that was *ex vivo* expanded with MPC (CB-AB006; clinicaltrials.gov NCT00498316).

CO-ADMINISTRATION OF CB AND HPDSCS

HPDSCs are largely non-adherent, minimally manipulated cell products derived from human placental perfusate depleted of RBCs. The CD34 content is approximately 2–6% and they have low CD4 and CD8 content, low class I and class II HLA expression and promote enhanced short-term and long-term engraftment with UCBT in non-obese diabetic/SCID animal model. Preclinical studies suggest the HPDSC may potentially facilitate UCBT

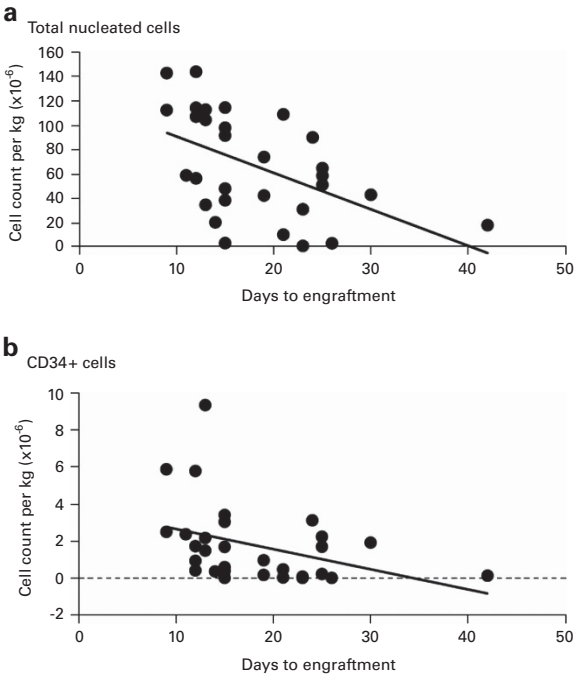


Figure 3. Correlation of total nucleated cells and $CD34^+$ cells with neutrophil engraftment.[18] In the units of expanded cord blood, the number of total nucleated cells per kilogram of body weight (**a**) correlated with the speed of neutrophil engraftment (Spearman correlation coefficient, −0.51; $P=0.004$), and the number of $-CD34^+$ cells per kilogram (**b**) also correlated with the speed of neutrophil engraftment (Spearman correlation coefficient, −0.48; $P=0.006$). Used from de Lima *et al*.[18]

engraftment, reduce severe acute GVHD and/or enhance immune reconstitution. Cairo *et al.* have initiated a pilot study of adding universal donor (third party) HPDSCs with either single or double UCBT following myeloablative or reduced toxicity conditioning in children and adults with selected malignant and nonmalignant diseases in a multicenter consortium (IND#14949; NCT 01586455; Figure 4). Fourteen children and adults have been entered in the study to date and there have been no adverse effects related to the HPDSC infusion, all with full engraftment and only 2 out of 14 have developed ⩾Grade II acute GVHD to date.[20] Additional correlative studies that are ongoing include donor chimerism, immune reconstitution and cellular immune recovery.[20]

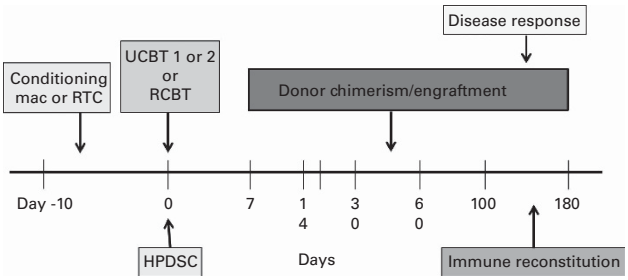


Figure 4. Experimental design (NYMC-550) CCT-HPDSC-UCBT-PI-001 (PI: MS Cairo, MD). A single-arm study to assess the safety of transplantation with human placental-derived stem cells combined with unrelated and related cord blood in subjects with certain malignant hematologic disease and nonmalignant disorders (NCT 01586455; PI: MS Cairo, MD). A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

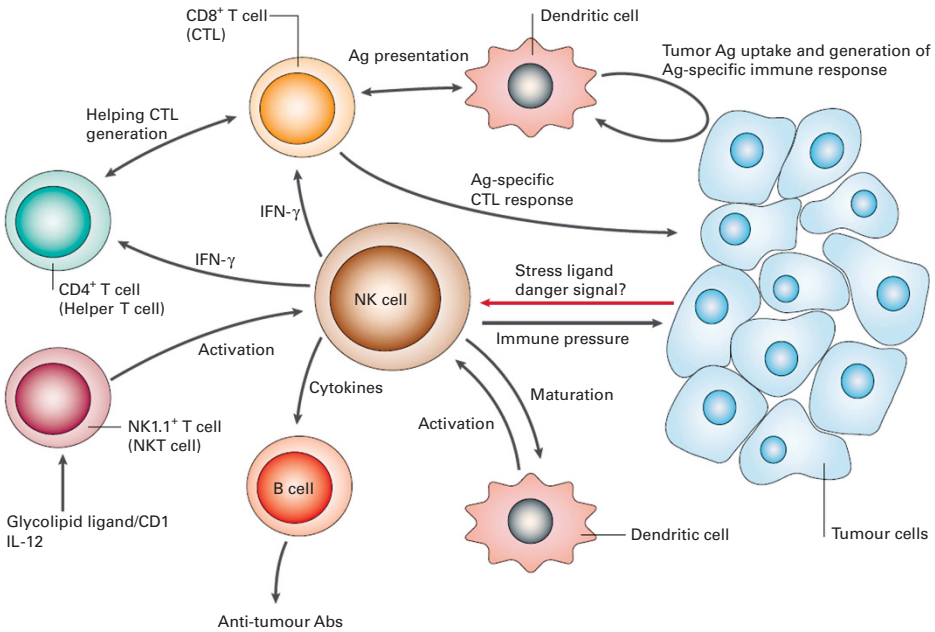


Figure 5. Central role of NK cells in tumor immunity.[50] The diagram shows a hypothetical scheme of the potential role of natural killer (NK) cells in tumor immune surveillance and in the network of immune cells that respond to tumors. NK cells might initially recognize certain 'stress' or 'danger' signals that are produced by tumors. Both NK cells and cytotoxic T cells (CTLs) are important mediators of antitumor immunity, as they are ultimately responsible for the destruction of the malignant cells. NK cells can influence the development of adaptive T- and B-cell immune responses that constitute specific immunity and immunological memory to tumors and pathogens. NK cell lysis of cancer cells could provide tumor antigens for dendritic cells (DCs), which induce them to mature and present antigen (Ag) to CTLs in lymph nodes. Cytokines, such as interferon (IFN)-γ, which are produced by activated NK cells, activate CTL and helper T-cell ($CD4^+$) responses. This leads to the proliferation of helper T cells and cytokine production. Activated $NK1.1^+$ T (NKT) cells can also induce the antitumour activity of NK cells. Cytokines that are produced by NK cells might also regulate B-cell production of antitumour antibodies (Abs). Reused with permission from Smyth *et al*.[50]

EX VIVO EXPANSION OF CB-DERIVED NK CELLS

NK cells are large granular lymphocytes characterized by the expression of CD56 and/or CD16 and lack of expression of CD3. They are not antigen specific and recognize transformed cells without prior sensitization. Target cell killing is regulated by the balance of inhibitory and activating cell membrane receptors that recognize self and danger signals, respectively, on the surface of target cells.[21] Inhibitory members of the killer cell immunoglobulin-like (KIR) and NKG2-family receptors recognize self HLA class I antigens, and activating receptors recognize stress ligands, viral proteins and antibodies on target cells. NK cell reconstitution after UCBT precedes T- or B-cell reconstitution by about 2 months (around day 30 vs day 100),[22] and is a critical in providing compensatory immune function in the face of T-cell reconstitution, which is further delayed in UCBT compared with marrow or peripheral blood HSCT.[23] In the early post-CBT period, NK cells preferentially express the inhibitory receptor NKG2A with reduced KIR expression, indicating that mature NK cells are present in low proportions.[24,25] Although these NK cells have high proliferative capacity and are functional against tumor cells, they exhibit higher interferon-γ production and reduced cytotoxic capacity compared with resting NK cells from healthy controls, which can be restored following cytokine exposure.[24,26,27]

The role of NK cells in engraftment following UCBT remains controversial. Gertow *et al.*[28] have suggested that mixed chimerism following double UCBT could possibly be related to NK cell tolerance between the CBU; other reports, however, did not show a correlation between KIR ligand incompatibility and engraftment.[29,30] Nonetheless, previous studies of hematopoietic stem cell transplantation in mice demonstrated that IL-2-activated NK cells mediate hematopoietic stem cell engraftment and that alloreactive NK cells may facilitate engraftment by killing recipient T cells and APCs.[31–33] As the reduced function and maturation of NK cells arising in the early post-CBT period can be restored by cytokines, infusion of *ex vivo* expanded and activated NK cells could represent a means to enhance early engraftment following UCBT.

Ruggeri and colleagues were the first to identify an antitumor role for NK cells in an HSCT setting in which they demonstrated that mismatch between donor and recipient HLA—with respect to their function as KIR ligands—resulted in lower risk of AML relapse.[33] Subsequently, KIR–KIR ligand mismatch has been correlated with improved outcome and decreased tumor relapse following allogeneic transplantation for patients with hematological malignancies.[34,35] This is mediated by direct effects on the tumor and multiple interactions with other immune cells (Figure 5). The role of NK cell-alloreactivity and leukemia relapse following UCBT has been controversial;[29,36] but early NK cell reconstitution is associated with improved disease-free survival and OS,[29,37,38] suggesting that augmenting NK cell recovery following UCBT would also decrease disease relapse and improve survival.

Purified and activated NK cells for adoptive transfer are easily manufactured, display high cytotoxic potential and carry a low risk of toxicity to the recipient. This strategy, however, is limited by the low numbers of NK cell available within CBU. To overcome these limitations, many groups have developed techniques for *ex vivo* expansion of NK cells adapted to UCB as the starting source to enable adoptive immunotherapy.[39–48] In addition, *ex vivo* IL-2-expanded NK cells from CB were shown to be active against AML blasts and showed anti-leukemia activity *in vivo* when infused into mice bearing human AML.[49]

NK cell-based cancer immunotherapy is an expanding scientific area of investigation. Further advances in the field will require increased knowledge of NK cell biology, models that predict donors or subsets with superior NK cell function, models that predict tumor susceptibility to NK killing and approaches for overcoming tumor resistance. Specific approaches under investigation include blocking ligand recognition by inhibitory KIR, combinations with immunomodulatory drugs and/or targeting antibody, selection or skewing of the NK cell repertoire, high-parameter phenotypic analysis of tumor ligands and imaging techniques to monitor NK cell distribution *in vivo* for understanding migration and homing.[50] Further research will be required to determine whether *ex vivo* expanded CB NK cells are effective in enhancing engraftment and preventing disease relapse. The infusion of NK cells expanded from CBU to augment immune recovery after HSCT is being investigated in clinical trials (clinicaltrials.gov NCT01619761 and NCT01823198).

SUMMARY

In summary, multiple approaches are being investigated to enhance hematopoietic engraftment, accelerate immunological reconstitution, reduce graft failure and transplant-related mortality, and increase OS following UCBT in children with malignant and nonmalignant diseases. Additional approaches not covered in this review that are under investigation to accelerate hematopoietic and/or cellular immune reconstitution following UCBT include *ex vivo* expansion of UCB with nicotinamide and the non-altered T-cell fraction, *ex vivo* enforced fucosylation with fucosyltransferase IV and guanosine diphosphate fucose, inhibition of dipeptidyl peptidase (DDP-4) by sitagliptin, *ex vivo* expansion with cytokines and StemRegenin 1 and the use of UCB-derived anti-viral CTLs are just a few of the newer contemporary approaches for *ex vivo* CB graft engineering being investigated at the present time.[51–56]

CONFLICT OF INTEREST

DAL has financial or ownership interest relating to *ex vivo* expansion of NK cells in Intrexon Corporation, Ziopharm Oncology and Cyto-Sen Therapeutics. The remaining authors declare no conflict of interest.

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