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Use of CB derived T-cells in cancer immunotherapy: milestones achieved and future perspectives

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Abstract

**Introduction:** Hematopoietic cell transplantation is a potentially lifesaving procedure for patients with hematological malignancies who are refractory to conventional chemotherapy and/or irradiation treatment. Umbilical cord blood (CB) transplantation, as a hematopoietic stem cell and progenitor (HSPC) source, has several advantages over bone marrow transplantation with respect to matching and prompt availability for transplantation. Additionally, CB has some inherent features, such as rapid expansion of T cells, lower prevalence of graft-versus-host disease and higher graft versus tumor efficacy that make this HSPC cell source more favorable over other HSPC sources. **Areas covered:** This review summarizes the current CB and CB derived T cell applications aiming to better disease control for hematological malignancies and discusses future directions to more effective therapies. **Expert Commentary:** CB transplantation could be used as a platform to extract cord blood derived T cells for *ex vivo* expansion and/or gene modification to improve cellular immunotherapies. In addition, combining cord blood gene-engineered T cell products with vaccination strategies, such as cord blood derived dendritic cell based vaccines, may provide synergistic immunotherapies with enhanced anti-tumor effects.

**Keywords:** Chimeric antigen receptor, Dendritic cells, Hematological malignancies, Immunotherapy, T cell receptor, Umbilical cord blood T cells
1. Introduction

Despite major improvements in the treatment and care of cancer patients in the last decades, cancer is still a disease with a critical unmet need for more efficient therapies. But for many malignancies, chemo- and radiotherapy are still the main first line treatment, which are often not sufficient to cure the patient, e.g. in aggressive forms of hematological malignancies, where the second option for curative treatment is hematopoietic cell transplantation (HCT) [1]. HCT transplantation allows the replacement of patient hematopoietic system by long-term reconstitution of a donor-derived hematopoietic system. Successful HSPC transplantation requires a human leukocyte antigen (HLA) matched donor, in first instance a sibling, which is only available for ~30% of patients [2]. Because matched donor cells are often not available, unrelated or haplo-identical donor cells are often selected, which can be retrieved from mobilized apheresis peripheral blood, bone marrow or cord blood.

Although allogeneic transplantation provides higher survival chances for chemo-resistant high risk hematological disorders, overall survival rate remains suboptimal (20-70% depending on disease/status) [3]. Hence, there remains an unmet need to further improve the survival chances. The availability of immune therapies has increased impressively in the last few years, which include adoptive cellular immunotherapy. Chimeric antigen receptor (CAR)-T cell therapy has entered clinical care in an increasing number of centers and some trials explore the potency of tumor-infiltrating lymphocytes in an autologous setting. Acquiring and expanding T cells from patients is technically demanding and clearly has its limitations. Alternatively, the use of ex vivo gene-modified T cells is a promising strategy, especially for treatment of hematological malignancies. Applications based on cord blood as a source to develop effective T cell immunotherapy are particularly interesting as it provides the use of adoptive T cell therapy in the context of cord blood HCT where the T cell product is being generated from the same graft used for transplantation. We here describe the possibilities of this approach.
2. Use of cord blood as a source for hematopoietic stem and T cell transplantation for hematological malignancies and immunotherapy

Cord blood (CB) is derived from the placenta and umbilical cord after childbirth. It has a different composition than peripheral blood and bone marrow (BM) [4], with more enriched primitive HSPCs [5] and a comparable proliferative capacity to HSPC-BM [6,7]. CB contains mainly naïve T cells [4], that can undergo rapid peripheral expansion with memory-effector differentiation within 4 weeks [8]. CB units have been stored in public or private CB banks [9], and are promptly available for transplantation, and it allows to use HLA mismatched unrelated donor cells up to 4/6 (high resolution (HR) typing) instead of 10/10 for bone marrow transplants, with reduced risk to develop graft versus host disease (GVHD)[10]. In the early days of CB transplantation, one of the main issues was the slower and delayed hematopoietic recovery and immune reconstitution that increased the possibility of infection-related mortality [11]. However, it has been shown that individualized dosing and timing of chemo- and/or serotherapy, such as anti-thymocyte globuline (ATG), can prevent rejection as well as GVHD and improves immune reconstitution. Low dose exposure of ATG improves T cell reconstitution, in particular CD4+ T cell recovery [12,13], which has been reported as a predictor for viral reactivations [14,15]. The CD4+-biased T-cell immune reconstitution, may be dictated by the distinct transcription profile similar to fetal CD4+ T cells [16], which are different from peripheral blood T cells. In an another study, CB CD4+ T cell characteristics were distinct from PB T cells in CD26 expression, and could produce higher interferon-γ and interleukin-5 expression after T helper (Th) type 1 and Th2 induced skewing [17]. These phenotypically distinct profiles of cord blood T cells may contribute to less viral infections even in the presence of low doses of ATG. Furthermore, the probability of relapse was found to be lower (40 vs 20%) after CB transplantation compared to the other groups [18]. However, although overall T-cell immune reconstitution in children is comparable after bone marrow transplantation or CB transplantation, CD8+ T-cell reconstitution is faster after bone marrow transplantation, whereas regulatory T cells (Tregs) and CD4+ T-cell recovery are faster after CB transplantation [3,19]. CD8+ T-cells have been shown to exhibit stronger proliferation and function after antigen-specific stimulation [20].
Even though CB can clearly provide advantageous responses in cancer treatment by inducing robust graft-versus-leukemia (GvL) responses [21] low numbers of tumor reactive T cells, especially during the early stages of immune reconstitution, still contribute to a considerable risk to develop relapses and reduced overall survival. To reduce these risks and provide lifelong curative therapies, the use of gene-modified T cells from CB is a promising strategy especially for treatment of hematological malignancies. A residual 20% of a cord blood unit is not infused into the patient; hence this can be used to generate a matched product by extracting cells for genetic modification to target tumor cells. T cell priming of reconstituting cord blood derived T cells or gene-engineered T cells can also be targeted in vivo by ex vivo engineered antigen-presenting cells from the same CB unit. This all underlines the potency to use of CB as a platform to develop adoptive T cell therapies in a CB transplantation setting or as off-the-shelf gene therapy products, which could also be used to treat patients with solid tumors (Figure 1).

3. Gene-engineered T cells

3.1 Cord blood derived CAR-T cell immunotherapy

Chimeric antigen receptor (CAR)-T cells are engineered T cells containing antibody domains coupled to co-stimulatory T cell specific co-stimulatory domains through a transmembrane domain [22]. Those cells are able to recognize surface antigens in a major histocompatibility complex class I (MHCI)-independent manner [23]. The production of CAR-T cells has been largely improved over the recent years. The first generation CAR-T cells were characterized by the presence of the antibody domain linked to the CD3-ζ transmembrane domain of TCR receptors. Second generation CAR-T cells are equipped with additional co-stimulatory motifs for T cell signaling, such as CD28 or 4-1BB [24]. However, the presence of 4-1BB seems superior, due to its ability to induce a central memory phenotype to T cells that persist longer (over two years) than solely incorporating a CD28 domain (∼30 days). The latest generation is called third generation CAR-T cells, combining more than one co-stimulatory domain to the CD3-ζ transmembrane motif. This could be of particular interest for cord blood T cells,
because 4-1BB and CD28 signaling play a synergistic role in redirecting UCB T cells against for instance B-cell malignancies [25].

The use of chimeric antigen receptor (CAR)-T cells is a promising strategy, that has recently resulted in significant successes in clinical trials targeting CD19 in chemo-refractory and relapsed B cell malignancies, particularly for acute lymphoid leukemia (ALL) [26,27]. CD19 is a B-cell specific surface antigen, highly expressed by the majority of B cell malignancies (80% of ALL, 88% of B cell lymphomas and 100% of B cell leukemias). CD19 it is also expressed on healthy B cells, which will be eliminated as well [28]. This could also play a role in the effectiveness of the therapy, because the CD19 CAR-T cells are strongly triggered due to the presence of high number of B cells, creating a strong immune response, also against the CD19 positive lymphoblasts. The loss of functional B cells can be counteracted by life-long immunoglobulin infusions. The FDA has recently approved the commercial use of Kymriah (Tisagenlecleucel), the first cell and gene-therapy based drug ever approved for the treatment of acute lymphoblastic leukemia (ALL) [29].

CARs contain antibody domains coupled to co-stimulatory T cell specific domains through a transmembrane domain, [22] to recognize surface antigens in a MHCI-independent manner [23]. Most of the trials are using CAR-T cells equipped with either CD28 or 4-1BB, important co-stimulatory motifs for T cell signaling [24]. However, the presence of 4-1BB seems superior, due to its ability to induce a central memory phenotype to T cells that persist longer (over two years) than solely incorporating a CD28 domain (~30 days). This could be of particular interest for cord blood T cells, because 4-1BB and CD28 signaling play a synergistic role in redirecting UCB T cells against B-cell malignancies [25].

To generate CAR T cells, CB T cells have advantages over the use of PBMCs, because they can be rapidly expanded and genetic modification of CB derived T cells could therefore be an effective approach. CB-derived T cells are amenable for transduction by lentiviral vectors to introduce CAR or recombinant TCRs. Several groups have produced impressive data regarding the efficacy of CD19-specific CAR CB derived T cells proving the cytolytic effect of CB derived and modified T cells both in vitro and in vivo in models of B-lineage acute lymphoblastic leukemia (B-ALL) [30,31]. Furthermore, in an ongoing clinical trial conducted by MD Anderson Cancer Center in Houston, Texas, gene-modified CB derived T cells
containing a CAR against CD19 were infused in patients with B-lineage lymphoid malignancies after umbilical cord blood transplantation (NCT01362452), providing evidence of the potential of CB T cells for cancer immunotherapy and especially in combination with CB transplantation.

3.2 Potential use of T cell receptor engineering for cord blood derived T cell immunotherapy

T cells use their T cell receptor (TCR) to recognize antigens of intracellular or extracellular proteins presented by MHCI or II on target cells. The first required signal for an effective T cell response is TCR activation followed by a series of co-stimulatory signals essential for the activation of intracellular pathways towards sustained responses. Tumor associated antigens (TAAs) that are selectively and/or overexpressed on malignancies can be used as target by engineered T cells with high affinity TCRs.

Hiwarkar et al. [16] demonstrated in a mouse model of B-cell lymphoma, the advantage of using CB derived T cells to increase graft versus tumor (GVT) effects significantly. Mice receiving CB T cells showed a reduction in the tumor growth that eventually resulted in complete regression. On the contrary, tumor growth continued in mice treated with PB T cells and untreated mice. No signs of GVHD were mentioned for up to 60 days in CB T cell transplanted mice, but in PB T cell transplanted mice severe signs of GVHD were observed three weeks after transplantation [16]. Effective therapy of CB T cells were also achieved by Lee et al. [32] in two mouse models of cervical and lung tumors. In those animal models, mice injected simultaneously with tumor cells and CB T cells did not develop any tumors as compared to control mice without CB T cells that showed normal tumor progression. Both these studies strongly supported that CB-derived T cells can eliminate cancer cells efficiently after infusion in tumor animal models.

The first clinical trial using TCR engineered T cells was published by Morgan et al. [33] in 2006 to treat melanoma patients, followed by trials for other types of solid tumors, such as esophageal, synovial sarcoma and colorectal cancer [33]. However, efficacy and safety of these therapies is often still suboptimal. There are intrinsic problems to some TCRs itself, such as low affinity towards the TAA, which could be increased by modification in the amino acid composition of the
crucial TCR-antigen-binding region [34]. Another problem has been that gene-modified TCR lose efficiency by mispairing with endogenous TCR α and β chains, whereby surface expression of the introduced TCR is decreased, and unwanted TCR reactivity against self-antigens can occur. This has been shown to risk lethal graft-versus-host disease in mouse models [35], which can be reduced by modifications that affect pairing between recombinant and endogenous TCRs, such inclusion of cysteine residues in the constant region of α and β chain [36] or by replacing human with mouse aminoacids in the constant domains [37]. The inherent nature of cord blood T cells to have reduced GVHD risk may also improve side-effects induced by mispairing of the introduced TCR with the endogenous TCRs. TAA-specific cytotoxic T cells (CTLs) were generated against multi-leukemia antigens, e.g. Wilms tumor 1 (WT1), human neutrophil elastase (NE) and melanoma-associated antigen A3[38]. It would be interesting to investigate if these TAA-specific CTLs could also be generated from cord blood T cells, because certain frequencies of TAA-specific T cells, in this case against PR1, are higher in cord blood than in adult blood [39]. Furthermore, engineered TCR can also be used to target virus specific antigen. Indeed, virus reactivation is one of the cause of death after HSPCT [14]. CB derived T cells against cytomegalovirus (CMV), Epstein Barr virus (EBV) and adenoviruses could be expanded in culture and reinfused to protect against reactivation of viruses. Several groups have shown that CB T cells can be robustly expanded ex vivo by using CD3/CD28 beads and cytokines, such as IL-2, IL-7 and IL-15 [40]. By using IL-7 and IL-15 naïve T cells could be directed towards long-living memory stem T cells (Tscm), that have the ability to self-renew and ability to turn into potent T cell effectors [41], which could be an excellent source to generate gene-engineered T cells. Hanley et al. demonstrated that in vitro priming of CB-T cells with APCs provided recognition of all three abovementioned virus antigens, but CB-T cells showed to have a larger pool of unconventional CMV epitopes compared to adult derived T-cells [42]. This strategy has been used recently in a clinical trial (NCT00078533) to increase the immunity against virus reactivation and the overall survival of patients. The CB-T cells were reinfused 30 days after the HSPC transplantation. Results have not been published yet. Cord blood T cells lines could also be generated that recognized multiple common viruses and at the same time provide antileukemic activity through the expression of a CAR targeting CD19, without causing GVHD [43].
Since most of the preclinical work based on recombinant TCR or CAR technology has been performed in PBMCs, more comparative work is required to test whether enhanced effects may be incurred by cord blood derived gene therapy products.

### 3.3 Generation of gene modified T cells from cord blood CD34+ progenitor cells

Another clinical approach of interest has been to obtain tumor-reactive T cells through *in vitro* generation of T cells, but this method has long been very inefficient. Culturing cord blood hematopoietic precursors on OP9 stromal cells that expresses Notch human ligand Delta-like1 showed that functional T cells with controlled antigen specificity in an HLA restricted manner could be obtained after transduction with TCR specific retroviral vector [44]. In another similar study, these cells also acquired additional natural killer cell-like killing of tumor cell lines. Furthermore, the *in vitro* propagation of retroviral TCR transduced CD34+ cord blood derived T cells also displayed little endogenous TCR expression with the tumor-reactive TCR highly expressed on the surface [45]. More recent reports show that human cord blood T cells could also be efficiently generated from HSPCs specific for cytomegalovirus (CMV) or Influenza-A virus epitopes without direct stromal co-culture or retroviral TCR transduction [46], which was also a tool to generate tumor-specific T cells against the antigen dopachrome tautomerase (hTRP-2) [47]. Using gammaretroviral vectors to introduce CAR or TCRs in CB CD34+ progenitor cells, van Caeneghem *et al* [48], demonstrated that endogenous TCR expression could be eliminated in most of the CD34 progenitor derived T cells, which could make this an excellent method to generate universal T cell products. Since cord blood HSPCs are highly amenable to lentiviral transduction [49], this may increase overall production of gene-modified tumor-specific T cells generated by this method.

### 4. *In vivo* priming of CB T cells: combinational therapy strategies

Priming of CB T cells could also be done directly *in vivo* by dendritic cells (DCs), which are an important determinant in the initiation of adaptive immune responses. These cells internalize antigens and present them to T cells, priming their activation [50] and mount CD4+ and CD8+ specific responses that can be used for cellular vaccines [51]. In fact, peripheral blood monocyte derived, or primary DCs
are used in several clinical trials and results suggest that patients are tolerating the infusion without showing severe side effects and with a good rate of short-term antitumor response. Most of the clinical trials are well summarized by Benteyn et al. [52]. However, this strategy requires a relatively high number of TAA-specific T cells present in the cancer patients to be efficient, which is usually not the case in hematological cancer patients after conditioning and subsequent HSPC transplantation. It takes time after a cord blood transplant for sufficient peripheral T cell expansion to occur and even longer to acquire sufficient numbers of T cells through thymic development. It can take months to obtain normal numbers of peripheral T cells. However, considering that DC vaccines can be used to stimulate CB derived and engineered T cells, this could be an interesting strategy for a combinational therapy to boost proliferation towards more tumor-reactive CB T cells.

This is even more interesting, because DCs vaccines can be generated from CB CD34+ progenitors. These cells have particularly shown efficient expansion properties and effective maturation into TAA specific DCs. De Haar et al. [53] developed a CB derived DC vaccine targeting Wilms’ tumor 1 (WT1) positive acute myeloid leukemia (AML)-blasts, which is an antigen overexpressed in the majority of AML patients. The CB derived DCs were able to efficiently migrate and activate WT1 specific T cells. We can speculate that this cellular vaccine should be able to boost both naturally occurring WT-specific T cells derived from cord blood as well as WT-specific engineered T cells. This strategy assures a perfectly matched product utilizing the same CB unit for both the transplanted donor cells, the produced DC vaccine and the CB engineered T cells. CB derived T cells can be primed against TAAs. Decker et al. demonstrated that chronic lymphocytic leukemia (CLL) patients-derived antigen presenting cells expressing CD40L are sufficient to prime CB T cells ex vivo, turning them into efficient CTLs able to kill CLL cells in vivo [54].

This shows the possibility to combine T cell and DC vaccine approaches base on a CB platform to mount superior immune responses against tumor antigens.

5. Strategies to improving the efficiency of CB-T cell therapy

To obtain a highly efficient immunotherapy product based on cord blood T cells, fine tuning of the specific molecular modifications related to tumor antigen
recognition are required but not sufficient. Experience in the field of T-cell immunotherapy demonstrated that some of the most promising in vitro products were not able to achieve the same efficiency in vivo.

The interaction between TCR and MHC I/peptide complex is by itself not enough to activate the downstream pathways to efficiently eradicate cancer cells and provide long-term memory. This requires sufficient co-stimulatory activation and no deleterious co-inhibitory effects. The expression of inhibitory signals has been shown to induce T cell exhaustion, senescence and anergy. One of the most studied inhibitory pathway involves the interaction between programmed cell death protein 1 (PD-1), expressed on T cells, and programmed cell death protein ligand 1 (PD-L1), usually expressed by APC or DCs, but often overexpressed on cancer cells[55]. The expression of PD-L1 has been described as a key factor limiting in the efficacy of adoptive cell therapies. Of note, the frequency of PD-1+ CD8+ T cells was significantly higher in patients transplanted with UCB and that experienced leukemic relapse, so there is a rationale to target this specific checkpoint inhibitor [56]. Another checkpoint inhibitor is cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4), which is another negative regulator of T cell immune function [57]. Therapies based on the use of blockade antibodies against those proteins lead to the suppression of the inhibitory pathway, increasing the survival and efficacy of CTL against the tumor [58]. In a specific cord blood humanized mouse model of Epstein-Barr virus (EBV)-induced lymphoma growth PD-1/CTLA-4 blockade markedly increased EBV-specific T cell responses, and enhanced tumor infiltration by CD4+ and CD8+ T cells [59]. In other mouse models using anti-Her-2 CAR T cells PD-1 blockade by anti-PD-1 antibodies boosted immune tumor-specific immune responses [60]. Instead of providing multiple checkpoint inhibitor antibody infusions, which also risk associated toxicities, T cells could be directly modified by gene-editing co-inhibitory genes. TALEN-mediated elimination of PD-1 expression on melanoma-reactive CD8+ T cells and in fibrosarcoma polyclonal T cells increased the persistence of T cells at the tumor site and increased tumor control [61]. In another study, CRISPR/Cas9 was used to eliminate PD-1 expression in CD19 CAR-T cells, which enhanced CAR T cell function significantly shows the potential of these applications,[62] which could also be applied on cord blood T cells. Other checkpoint inhibitors may also be of interest depending on its expression on tumor or gene-modified T cells. Although more proof-of-principle studies are reported on the use of gene-editing systems,
such as CRISPR/Cas9, since it is relatively novel in this field, identifying off-target
cleavage events by genome-wide non-biased screening is important [63], as well as
developing efficient Cas9 nucleases with less off-target cleavage sites [64]. This
could particularly pose a risk in multiplexing gene-modified T cells.

Another approach to generate a universal off-the-shelf immunotherapy products is
to eliminate endogenous TCRs, independent of MHC expression in the host, has
been tested a few years ago by using zinc-fingers to target the α and β constant
domains of TCRs [65]. These initial methods using zinc-fingers had relatively low
efficiency. Another approach that followed was the use of TALEN to engineer
CD19 T cells [66] and to eliminate expression of TCRα to make to product
universal and delete CD52 to protect the cells from alemtuzumab treatment. These
non–human leukocyte antigen–matched donor peripheral blood mononuclear cells
(PBMCs) were infused into two infants with relapsed refractory CD19⁺ B cell
acute lymphoblastic leukemia that received lymphodepleting chemotherapy and
alemtuzumab serotherapy to successfully bridge to allogeneic stem cell
transplantation [67]. More recently, effective CRISPR/Cas9 mediated insertion of a
CD19-specific CAR into the TCR α constant (TRAC) locus resulted in uniform
expression and also enhanced T cell potency compared to conventionally generated
CAR T cells, showing promising prospects for gene-edited CAR T cells products
[68].

Of note, the production of universal TALEN gene-edited CAR T cells by
CliniMACS magnetic bead selection for TCRαβ T cells still resulted in
approximately 1% TCRαβ positive T cells that were infused in two B-ALL
patients [67]. In one of these patients grade 2 skin GVHD was confirmed, derived
from the small infused TCRαβ positive T cell population. The use of CB derived T
cells as a source to make universal CAR T cells may further lower the risk of
GVHD.

6. Cord blood T cell immunotherapy: opportunities and limitations

Although immunotherapy is showing promising results, there are still several
problems that have not been addressed. In primum, the success achieved with those
strategies in hematological malignancies has not been reproduced for solid tumors.
TCR engineered T cells can be strongly affected by the downregulation of MHC
class I, a strategy often adopted by tumor cells in order to avoid immune surveillance [69]. Martini et al. also demonstrated that MHCI expression can be increased by use of IFNγ in a mouse model of prostate cancer [70], but the translation can be risky, considering that IFNγ can be toxic. On the other side, since CAR-T cells are HLA independent, this could be an advantage for the treatment of solid tumors. Lately, clinical trials using CARs are also focusing on other malignancies, such as prostate and pancreatic cancer, glioma, neuroblastoma and sarcoma [33,71,72]. However, up to date, the two most positive trials reported are one targeting neuroblastoma, with three of eleven patients with complete remissions [73], and sarcoma, with four of seventeen patients showing stable disease [74]. Those limited results are mostly due to the immunosuppressive microenvironment, which is difficult to reach and attack by T cells [72]. Two other major problems are on-target [75] and off-target toxicity [76] with a risk of severe multi-organ failure. Optimized dosing after infusion of those cellular gene-modified products for predictable pharmacokinetics and pharmacodynamics is still largely unknown, but will be required to obtain maximal responses, but also to reduce the observed side effects and toxicity [77]. The most serious side-effect observed in CAR-T cell therapy was cytokine release syndrome (CRS), experienced by almost all the B-ALL patients treated [78]. Fortunately, CRS can be controlled with anti-IL6R antibodies to reverse the symptomatology, but the patients need to be strictly monitored [79]. Another problem is the manufacturing process of peripheral blood TCR or CAR T cells, which is generally slow and expensive but also results in batch to batch variability. Currently, a lot of effort is invested in optimizing the manufacturing process by automating the enrichment, transduction and expansion of gene-modified T cells to improve quality and reduce costs [80]. Automated manufacturing of immunotherapy products in closed systems should widen applicability also for cord blood off-the-shelf products [81], especially considering the high availability of CB unit stored in the CB biobanks. Cord blood units could be potentially selected containing high numbers of T cells or specific subsets to be prepared for off-the-shelf products. The number of T cells in a cord blood unit may be limiting, and ex vivo expansion to create larger numbers may aid in efficacy of treating cancer. Additionally, storage of small numbers of T cells needs to be assessed to provide high quality recovery of for infusion into patients. Efficient recovery of functional HSPCs from long stored cord blood units have been reported (21-23.5 years) [82].
al, demonstrated that cryopreservation has no effect on natural killer cells expanded in vitro from CB-CD34+ [83]. Moreover, functional CB derived T cells can be recovered, but these frozen products contain apoptotic cells due to the freeze/thaw procedure. It has been demonstrated the overall percentage of viable T cells can be increased in cultures containing IL-7 during expansion [84]. In a transplantation setting, T cells can be derived from the 20% portion and gene modified and expanded. For off-the-shelf purposes, a whole CB unit can be used to obtain a larger pool of CB derived T cells.

In order to improve the efficiency of T cell immunotherapy for the treatment of solid tumors it may also be important to increase homing to the tumor niche. In the first steps of this process, T cells may be hampered to efficiently home to the tumor environment due to interference with attractive chemokines, i.e. mainly chemokine (C-X-C motif) ligand (CXCL) 9, 10 and 11 [85] and/or production of vascular endothelial growth factor (VEGF) [86,87]. An example to improve trafficking and infiltration is supplementation of attractive chemokines or use VEGF inhibitors [88,89]. Additionally, lack of MHC I, as mentioned before, can be crucial in determining the efficiency of those therapies. For example, when the downregulation of MHC I is due to epigenetic modification resulting in DNA hypermethylation, the expression of the protein is suggested to be reversed using histone deacetylase (HDAC) inhibitors, such as Vorinostat [69]. Consequently, it is also important to modify the immunosuppressive tumor microenvironment, characterized by inhibitory cells or soluble factors released by the tumor, able to decrease the efficiency of T cells immunotherapy products [72,90,91].

For future implementations better understanding of the environmental tumor biology is important to identify adjuvant strategies for the most optimal effect of CB-T cell immunotherapeutic products. CB-derived T cell therapies can be used in the HCT transplantation setting for treatment of hematological malignancies in the minimal residual disease stage to provide immunological memory to fight future relapses. In this case the T cells can be generated from the graft to ensure physiological memory formation. Alternatively, T cells can be used as an add-on cellular therapy to treat patients with progressed or established relapsed disease. For the latter, off-the-shelf cell therapies are an attractive possibility.

7. Conclusion
We discussed the developments of state-of-the-art T cell immunotherapy using CB cells, which are relevant to cord blood T cell applications aiming for better disease control in patient with malignant indication. Especially for gene modification of T cells, there is a clear need for improvement before developing it into a standard therapeutic practice. We focused on the advantages of using CB derived T cells above other cell sources as target cell for genetic modification for the future cell therapy products. The unlimited availability, making manufacturing of off the shelf products easier, the highly proliferative capacity and naïve phenotype makes these cells of special interest above other cell sources. In addition, it makes it easier to create standardized products.

Primarily it is of particular interest for hematological malignancies in combination with HCT transplantation, but also manufacturing of off the shelf products is of interest. Optimization of automated protocols should decrease batch to batch variability and improve the quality of the gene-therapy products. The presence of large CB biobanks provides an opportunity to also create effective “off-the-shelf” immunotherapeutic products, ready to be rapidly infused when required, and which can also be used for non-hematological related malignancies. To obtain improved immunotherapy products, homogeneity between hospital centers, cohorts, and especially manufacturing strategies needs to be optimized.

Combining immunotherapeutic gene-modified products can increase the chance of overall survival by consequently decreasing relapse rate and infection related mortality. However, more comparative studies are required, as well as further confirmation and optimization of its potential applications, with the aim to develop superior “off the shelf” CB derived immunotherapy products.

8. Expert Commentary

Optimal immune reconstitution is required to prevent relapses and improve overall survival of patients with hematological malignancies after HST. This underlines the importance of personalized dosing of conditioning drugs and cellular immunotherapy products to provide optimal immune reconstitution. Optimal tuning of immunomonitoring should assist in the development of therapies that target leukemic cells, in particular combinational therapies which are required for effective anticancer treatments to prevent minimal residual disease, relapses and
completely eradicate all tumor cells. This goal could be reached with the use of immunotherapy products consisting of engineered T cells derived from the peripheral blood of patients. The gene-therapy field has made considerable advancements in the last decade, which has resulted in gene-vehicles that can efficiently deliver CARs and TCRs to T cells to provide specific antitumor responses. This strategy has already been investigated especially for eradicating CD19+ ALL. However, the manufacturing process for producing personalized cellular drugs is still far from optimized and has not given standardized results yet. The use of cord blood T cells as targets for gene-modified immunotherapy products have advantages, because of their potential expansion and reduced risk of graft versus host disease. This may provide safer gene-therapy products when recombinant TAA-specific TCRs are introduced. It is of particular interest that the research field has advanced in the generation of T cells derived from cord blood CD34+ progenitor cells. Since these cells maintain their TCRαβ germline loci after introduction of a recombinant TCR, this also significantly reduces TCR mispairing, hence improving safety by lowering the risk of autoimmune reactivities. To use this as a broad application in cancer patients, it is important to expand the available antitumor specific TCRs to cover more HLA-types, hence more of these TCRs need to be retrieved from cancer patients or from in vitro TCR selection methods. Furthermore, multiple TAA-specific TCRs are also required to limit the risk of tumor-evasion of T cells, which may be limited through combining multiple TAA-specific T cells in a single cellular therapy. CD34+ progenitor cord blood derived T cells could be especially useful for preparation of off-the-shelf products, because very low numbers of cells are necessary to create billions of TAA-specific T cells through in vitro expansion. More recently, especially with the development of TALEN and CRISPR/Cas9 mediated gene-editing, improved efficiencies to modulate or eliminate gene expression has created possibilities to bypass tumor strategies to evade immune surveillance and thereby to enhance antitumor responses. Since these gene-editing tools have been recently developed thorough safety assessment is required to predict potential side-effects of applying these tools for cellular anticancer gene therapy products. The potential effects of off-target gene-editing are still largely unknown and unpredictable, especially in an approach to target multiple genes at once. Whole-genome sequencing is therefore important to accurately assess the off-target effects, and to develop algorithms to predict single and multiplex gene-
editing off-target cleavage sites. Additionally, the elimination of checkpoint inhibitors may also potentially lead to autoreactivity due to uncontrolled inhibition after TCR stimulation.

The applications of these tools to cord blood derived cells, in particular to cord blood T cells in a setting after UCB transplantation should be investigated for its feasibility and safety, and whether combining these strategies with other products derived from cord blood, such as dendritic cell vaccines, should lead to superior next-generation treatments of hematological malignancies.

9. Five-year view

UCB transplantation is a commonly accepted practice for patients with hematological malignancies who are refractory to conventional chemotherapy and/or irradiation treatment. More knowledge will be gathered over the next few years to predict the required personalized dosing to provide optimal immune reconstitution. In the recent years, CB derived dendritic cell vaccines have been developed to boost T cell responses after transplantation. Because cord blood T cells can be retrieved from cord blood banks, personalized and/or universal products can be developed, that are gene-modified to specifically target tumor-antigens. Modifications on the genomic level through gene-editing also provide opportunities to modulate inhibitory signals to enhance antitumor effects. Initial results on PBMC T cells have shown that gene-engineering and multiple genomic modifications are feasible, and it is expected that this will lead to more multiplexed genome-edited anticancer cellular products. These modifications applied on PBMC T cells can be transferred to cord blood derived T cells. Ex vivo expansion and/or gene modification of these cord blood T cells can potentially lead to improved cellular immunotherapies. Recent improvements in the development of CD34+ derived cord blood T cells should lead to GMP protocols and possible translation to clinical applications. Combining these cord blood gene-engineered T cell products with cellular vaccinations, such as cord blood derived dendritic cell based vaccines is anticipated to enhance immunotherapeutic anti-tumor effects.

10. Key issues
● Personalized dosing of conditioning drugs in umbilical cord blood transplantation settings is required to provide optimal immune reconstitution.
● CB derived T cells mediate enhanced antitumor effects compared to adult peripheral blood T cells.
● CB derived T cells can be rapidly expanded *ex vivo* and genetically modified. Their naïve phenotype can be directed to long-living memory stem T cells, which may provide improved antitumor effects.
● Recently developed gene-editing techniques, such as TALEN or CRISPR/Cas9 can be exploited for multiplex gene-editing to improve T cell antitumor responses, which can also be applied to cord blood T cells.
● Umbilical cord blood units can serve as a platform to generate superior “off the shelf” CB derived immunotherapy products that can be used to potentially boost antitumor immune responses for more effective therapies. CD34⁺ progenitor cord blood derived T cells could specifically serve as target cells to create large numbers of TAA-specific T cells with reduced risk of autoreactivity providing an alternative source for generating off-the-shelf antitumor products.
● Automated manufacturing of immunotherapy products in closed systems, including enrichment, transduction and expansion of gene-modified T cells to improve quality and reduce costs, should widen applicability also for cord blood off-the-shelf products, especially considering the high availability of CB unit stored in the CB biobanks.

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**Declaration of interest**
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References

Papers of special note have been highlighted as:
* of interest
** of considerable interest


* Key paper that shows improved antitumor responses by cord blood T cells.


* Severe side-effects caused by mispairing of endogenous TCRs with introduced recombinant TCRs.


* Modulation of Cas9 proteins to reduce off-target cleavage and increase specificity.


** First report to use multiplexed TALEN mediated gene-edited CAR T cells to effectively eradicate leukemia cells and bridge to allograft transplantation.
** Locus specific integration to control expression of a CAR and enhance anti-tumor effects.


Figure 1. Combinational cellular immunotherapy based on umbilical cord blood. Schematic representation to combine cord blood transplantation (CBT) with immunotherapy products derived from the same cord blood unit. The remaining 20% of a CB unit that is not transplanted can be used to develop CD34+ derived dendritic cells (DCs) or TCR gene-engineered T-cells. These strategies can also be used as mono-therapy or combined to enhance the immunotherapeutic effect.