Expansion or Incubation? Which is Better to Manipulating Stem Cells for Improving the Effects of Umbilical Cord Blood Transplantation

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Abstract

**Background:** Delayed hematopoietic recovery was one of the factors that limited the clinic application of umbilical cord blood (UCB) transplantation. Two different methods of overcoming the limitation were evaluated by this systematic review and meta-analysis. One strategy was to expand UCB unit *ex vivo* for increasing cell dose and another strategy was to incubate UCB unit with small molecules *in vitro* for enhancing bone marrow (BM) homing ability.

**Methods:** Relevant literatures were collected from 2000 to 2020 by searching the databases of PubMed, Medline, and EMBASE. A total of 15 studies that met the inclusion criteria were included in the meta-analysis.

**Results:** Similar clinical effects including neutrophil recovery (P=0.308), platelet recovery (P=0.4754), and 100-day survival (P=0.4216) between two methods were observed. Faster neutrophil recovery was associated with higher infused total nucleated cells (TNCs) (R²=0.4526 P<0.05) and higher infused CD34+ cells (R²=0.9777 P=0.095) in patients of expansion group and incubation group, respectively.

**Conclusion:** Although the treatment outcomes by using expanded stem cells were getting better all these years with fast development of cell culture techniques according to our cumulative meta-analysis. It seems the application of incubation procedure was safer and more convenient than expansion regarding to their similar clinic effects. This study was registered at RROSPERO (Registration no. CRD42020149750).

**Background**

The field of umbilical cord blood banking and transplantation has grown exponentially over the last 25 years. Receiving umbilical cord blood transplantation (UCBT) may be a good choice for hematological malignancy patients when the HLA-matched donors are not available or the transplantations are urgently needed [1]. Despite the encouraging results have been gotten from pediatric patients, several disadvantages of UCBT have emerged for adults. For instance, the high risk of graft failure, delayed hematopoietic recovery, and poor immune reconstruction, delays the pace of UCBT clinical application. The sparsely-numbered of stem cells and mature immune cells in a single UCB unit could be an explanation for these shortcomings [2]. To make UCBT as effective as bone marrow transplantation and peripheral blood transplantation, different stem cell manipulation methods have been used.

Expanding UCB cells *in vitro* for several days is a widely-used method to increase the numbers of TNCs and CD34+ stem cells. Theoretically, with a perfect expanded process, an ideal treatment outcome can be achieved in a single UCB unit transplantation. And double UCB units’ transplantations have paved safer ways for the applications of UCB cell expansion products by avoiding the risks of cell culture or graft failures in single unit UCBTs [3, 4]. Another way is to enhance the UCB stem cells’ bone marrow homing ability by incubating them with small molecules within a short time. The neutrophil and platelet recovery can be accelerated and the survival can be prolonged with the application of incubated products,
although the UCB doses are not altered during manipulation processes [5]. Other methods have also been studied in varying extent, such as the co-infused HLA-mismatched UCB expansion products [6, 7], intra-BM injected donor cells, and co-infused donor T cells [8, 9]. Notably, only the first two methods were evaluated in our study, whereas the rest were beyond the scope of our research objects. The similarities and differences between two kinds of manipulation will be studied in our systematic review and meta-analysis.

**Method**

This study had been registered at http://www.crd.york.ac.uk/prospero/ (Registration no. CRD42020149750). And the study were designed by the statement of preferred reporting items for systematic reviews and meta-analyses (PRISMA) [10].

**Search strategy**

The PubMed, Medline and EMBASE database were searched for the period from 2000 to 2020. We used the searching terms “improve engraftment” and “ex vivo expansion or incubation” in combination with “umbilical cord blood” and “transplantation”.

**Study selection**

Since no proper clinic trial that directly compared these two methods was available, we therefore included studies that evaluated one of the two methods provided they had the same inclusion and exclusion criteria. The enrolled studies must met the following criteria: (a) the UCB units chosen for manipulation were required to be ≥ 4/6 human leukocyte antigen (HLA)-allele matched with the recipients; (b) in double UCB units’ transplantation cases, the manipulated UCB unit must be infused to patients together with the unmanipulated one at the same time (twenty-five unmatched patients in one study [11] were excluded while the other twelve remained); (c) the neutrophil recovery time need to be accurate and available in articles, with or without the historical control data. (d) no duplicate reported results.

**End points**

Primary endpoints of this study were the neutrophil and platelet recovery time after UCBT. Secondary endpoints were 100-day survival rate. The relationship between infused cell doses with transplantation outcomes were also evaluated.

**Quality assessment**

The quality of included studies were assessed by reporting the key points of the clinic trials’ designs [12]. Such as the number of infused UCB units per patient; the stem cell selection procedures before expansion *ex vivo*; cytokines and mediums that were used for manipulation; days required for expansion or incubation; number of patients; the medium weight and age of patients and the conditioning methods.
**Statistical analysis**

The outcomes of historical controls were not available in some studies. To be more accurate and avoiding published bias, we used the STATA version 12.0 to make a single-arm meta-analysis. Outcomes were compared using the reported means and standard deviations (SD). If the data was not available in literature, we would ask for it from the author directly. And the data could also be estimated from the reported median and ranges without author’s response [13]. A random effects model will be chosen with the $I^2 > 50\%$.

**Results**

**Literature search**

The procedure of study selection was presented in Fig. 1. A total of fifteen clinic trials were included at last. Twelve of them were designed to study the effects of stem cells expansion *in vitro* while the other three were focusing on the incubation of stem cells *ex vivo*.

**Study characteristics**

Fourteen phase I/II clinic trials [11, 14–26] and one phase II/III clinic trials [27] were included and their characteristics were presented in Table 1. One study [14] had a randomized control group while fourteen studies [11, 15–27] were single arm clinic trials for using historical controls. It’s worth mentioning that the historical controls were well matched for transplantation conditioning, disease status, age, graft size, HLA matching and performance score criteria. Children and adult patients were both included in eight studies [11, 15–17, 21, 24, 26, 27] while six studies [18–20, 22, 23, 25] only focused on adult patients. Twelve studies [11, 15, 17, 18, 20–27] used myeloablative conditioning whereas only one study [19] used nonmyeloablative regimens, and two [14, 16] used both of them. One manipulated UCB unit and one unmanipulated unit were co-infused to patients in eleven studies [14, 16–25]. Four studies [11, 15, 26, 27] used only one manipulated UCB unit. Only one study didn’t select stem cells with CD34 + or CD133 + biomarker before expansion *in vitro* [18].
Table 1
Characteristics of studies included in the meta-analysis.

| Study        | Phase | Patients (experimental) | Patients (control) | Age                  | Preconditioning | CBUs | Expanded cell type |
|--------------|-------|-------------------------|--------------------|----------------------|-----------------|------|-------------------|
| 2002 Shpall  | I     | 37                      | -                  | children/adult       | MA              | 1    | CD34+             |
| 2007 Lima    | I/II  | 24                      | 24                 | -                    | NMA/MA          | 2    | CD133+            |
| 2008 Lima(BMT) | I/II  | 10                      | -                  | children/adult       | MA              | 1    | CD133+            |
| 2008 Lima(Blood) | I/II  | 35                      | 36                 | children/adult       | NMA/MA          | 2    | CD133+            |
| 2010 Delaney | I     | 10                      | 20                 | children/adult       | MA              | 2    | CD34+             |
| 2012 Lima    | I     | 31                      | 80                 | adult                | MA              | 2    | unselected        |
| 2013 Delaney | I     | 23                      | 40                 | children/adult       | MA              | 2    | CD34+             |
| 2014 Horwitz | I     | 11                      | 17                 | adult                | MA              | 2    | CD133+            |
| 2016 Wagner  | I/II  | 17                      | 111                | children/adult       | MA              | 2    | CD34+             |
| 2017 Anand   | II    | 19                      | 86                 | adult                | MA              | 1/2  | CD133+            |
| 2018 Stiff   | II/III| 101                     | 295                | children/adult       | MA              | 1    | CD133+            |
| 2018 Horwitz | I/II  | 36                      | 146                | children/adult       | MA              | 1    | CD133+            |
| 2013 Brunstein| I     | 29                      | -                  | adult                | NMA             | 2    | -                 |
| 2015 Popat   | I     | 22                      | 31                 | adult                | MA              | 2    | -                 |
| 2013 Cutler  | I     | 21                      | 53                 | adult                | MA              | 2    | -                 |

Procedures of stem cell manipulation
The manipulation procedures of UCB units have been summarized in Table 2. The effects of expansion or incubation determined the cell status such as cell number, cell viability and cell type. And the outcomes of UCBT were closely associated with the cell status. Most of the expansion studies added regular cytokines into their cell culture systems including stem cell factor (SCF), FMS-like tyrosine kinase 3 ligand (Flt3L), megakaryocyte growth and development factor (MGDF) were also used in early studies [11, 14]. However, the effects of regular cytokines cultured stem cells on speeding up time to neutrophil recovery were controversial [28–31]. Some new small molecules or feeder cell layers were added into the cell culture system to augment the effects of regular cytokines in later expansion clinic trials [18, 24].
Table 2
Details of manipulation methods.

| Study               | Cytokines          | Additional intervention | Culture time |
|---------------------|--------------------|-------------------------|--------------|
| 2002 Shpall         | SCF GCSF MGDF      | -                       | 10 d         |
| 2007 Lima           | SCF GCSF TPO       | -                       | 14 d         |
| 2008 Lima (BMT)     | SCF TPO Flt3L IL-6 | 5uM TEPA                | 21 d         |
| 2008 Lima (Blood)   | SCF GCSF TPO       | -                       | 14 d         |
| 2010 Delaney        | SCF TPO Flt3L IL-6 IL-3 Delta1<sup>ext</sup>−IgG |                      | 16 d         |
| 2012 Lima           | SCF TPO Flt3L IL-6 GCSF | MSC                   | 14 d         |
| 2013 Delaney        | nr Delta1<sup>ext</sup>−IgG | nr                  |              |
| 2014 Horwitz        | SCF TPO Flt3L IL-6 | 2.5 mM NAM              | 21 d         |
| 2015 Forcade        | SCF FLT3L TPO GCSF | -                       | nr           |
| 2016 Wagner         | SCF TPO Flt3L IL-6 | SR-1                    | 15 d         |
| 2017 Anand          | SCF TPO Flt3L IL-6 | 2.5 mM NAM              | 21 d         |
| 2018 Stiff          | SCF TPO Flt3L IL-6 | 5uM TEPA                | 21 d         |
| 2018 Horwitz        | SCF TPO Flt3L IL-6 | 2.5 mM NAM              | 19–23 d      |
| 2013 Brunstein      | - 1 µg/m C3aL      |                         | 15 min       |
| 2015 Popat          | - 1 mM β-fucose   |                         | 30 min       |
| 2013 Cutler         | - 10uM PEG2       |                         | 60 min/120 min |

As for incubation studies, the cytokines mentioned above were not necessary. Three kinds of small molecules were used to improve the stem cells’ homing ability by incubating with UCB units from fifteen minutes to two hours. And the incubating time was vital. PGE2 could contribute to a better clinic outcome through two hours’ incubation with stem cells, however, the effects were not good as only one hours’ incubation. So the data of patients in one hours’ incubation group were not evaluated in our study.
Comparing the differences between two methods, the processes of UCB expansion often cost lots of materials including serum free mediums and cytokines. All these additive is known to be expensive and could increase the financial burden on patients at last. There is also high risk of contamination during the several days of cell culture. In fact, the contamination cases are often reported in expansion studies [22, 24]. As a result, the incubation procedures seemed to be cheaper, safer and more convenient in accelerating neutrophil recovery.

Described in Table 3, expansion procedures yielded an average of 1.7–854 fold expansion (FE) in TNCs and 2.2–330 FE in CD34+ cells. Manipulated UCB units had more TNC numbers than unmanipulated units. The difference was larger for CD34+ cells. As a comparison, incubating did not alter the cell numbers. Much more TNCs and CD34+ cells thus could be infused to patients after expansion manipulation. But can they lead to a better outcome in UCB transplantation?

Neutrophil recovery

The medium time and range of neutrophil recovery can be acquired from fifteen studies [11, 14–27] and the data of randomized or historical controls are available in ten studies. Although the evaluation including data from both treatment and control group is the gold standard for meta-analysis, more published bias will emerge with only these ten studies included. To make a more rounded analysis, we discreetly enrolled all fifteen studies including five studies that didn't have control groups and regarding all of them as single-arm clinic trials. And the other outcomes will be evaluated in the same way.

The overall effect size (ES) of neutrophil recovery in expansion studies and incubation studies was 17.77 (95%CI 14.02–21.51) and 16.87 (10.04–23.70), respectively (Fig. 2A-B). No significant difference was found (P = 0.308). Expansion or incubation, compared with control group, could both accelerate the neutrophil recovery. But it seems the time to neutrophil recovery remained the same even these patients in expansion studies received much more TNCs and CD34+ cells.

Platelet recovery

The Overall ES was 47.83 (95%CI 38.80-56.86) for expansion manipulation group and 46.03 (95%CI 38.58–53.48) for incubation manipulation group. (Fig. 2C-D). No significant difference between two kinds of manipulation was found (P = 0.4754). The result revealed that the patient's platelet recovery time in expansion groups was barely shortened after being infused so many expanded UCB stem cells comparing with the patients in incubation group.

100-day survival

Based on existing studies, eight expansion studies and two incubation studies reported the data of 100-days survival rate. The overall ES of 100-days survival rate was 0.91 (95%CI 0.84–0.97) for the expansion studies and 0.89 (95%CI 0.79–0.97) for the incubation studies. No significant difference between two manipulation procedures was found (P = 0.4216) (Fig. 3).
Relationship between cell dose and outcomes

The association between UCB cell dose and hematopoietic recovery was assessed by a list of studies [32–34]. The positive correlations between the two factors were reported, although not all of them were significant. This was further confined by a systematic review [35]. In our enrolled studies, Wagner et al, de Lima et al, and Horwitz et al reported the strong correlation between neutrophil recovery and infused TNC or CD34 + cell dose [18, 22, 24]. A moderate negative association between total infused TNC dose and neutrophil recovery in expansion studies was revealed ($R^2 = 0.4526 P < 0.05$). The total TNC dose was unrelated to the neutrophil recovery in incubation studies. We also found a moderate correlation between CD34 + cell dose and neutrophil recovery in incubation studies ($R^2 = 0.9777 P = 0.095$), however, not in expansion studies. Unlike the reported results in other reviews, no observable correlation between platelet recovery time and cell dose was revealed.

Mechanism involved in manipulation

The function and cell number of UCB units were believed to be altered by the small molecules and feeder cells. Not surprising, the involved intrinsic and extrinsic signals were different between two methods. And some common signals shared between expansion and incubation were also revealed (Fig. 4).

Delta-1$^{\text{ext-IgG}}$, mesenchymal stem cells (MSC), SR-1, copper chelator, and nicotinamide (NAM) were used for stem cell expansion ex vivo. Hes-1, known as a Notch transcriptional target, could preserve the long-term reconstituting hematopoietic activity and the self-renewal ability of stem cells [36–40]. And the clinic trials could be able to be conducted with the application of immobilized Delta-1 ligand [41]. MSCs have been widely used for stem cell expansion as feeder layers [42–45]. As for the mechanisms that may be responsible for MSC’s regulating effects, no all agreed explanation was achieve. Y. K. Jang et al considered that a list of cytokines including IL-1α/β, IL-6, IL-7, leukemia inhibitory factor (LIF), FL, TPO, SCF, C-kit, macrophage colony stimulating factor (M-CSF), and GM-CSF that secreted by MSCs could support the ex vivo expansion of stem cells [46]. High level of SDF-1α, an effective chemotactic factor which contributes to stem cell bone marrow homing, was also detected in the supernatants of UCB expansion system [47]. SR-1 could inhibit differentiation and promote proliferation of stem cells by antagonizing aryl hydrocarbon receptor (AhR) [48–50]. Copper chelator could reduce the intracellular copper content of stem cells by decreasing mitochondrial membrane potential, which lead to a better expansion result [51–54]. NAM could reduce the p21 expression and promote the proliferation of stem cells as one of the noncompetitive inhibitors of SIRT1. The homing ability was also increased by the regulation of CXCR4 downstream signaling pathways [55, 56]. Prostaglandin E2 (PGE2), C3a, and fucosylation were used for short time incubation to increase the bone marrow homing ability. PGE2 could upregulate the expression of Cox1 and Cox2, which contributes to stem cell proliferation [57]. The homing ability of stem cells was also increased by up-regulating CXCR4 after PGE2 incubation. Additionally, survivin, as the inhibitor of intracellular active caspase-3, was activated by PGE2 and thus suppressed the apoptosis of stem cells [58–60]. C3a-C3aR axis plays a functional role in enhancing marrow homing ability of stem cells by increasing their responsiveness to SDF-1. And the matrix metalloproteinase-9
(MMP-9) was involved in this process [61, 62]. Treatment of stem cells with the enzyme fucosyltransferase-VI and guanosine diphosphate fucose could increase the cell-surface sLeX determinants, further enhancing homing ability of stem cells through a better P- and E-selectin binding capacity [63–65].

Ongoing clinic trials

There are a total of seven clinic trials ongoing and all of them are using ex vivo expansion methods (NCT01590628, NCT01690520, NCT02730299, NCT03173937, NCT03441958, NCT04103879, NCT03913026). No clinic trial about incubation is available. Obviously, clinicians prefer expansion to incubation. Their belief might rely on the continuous development of cell culture techniques and application of effective new molecules, such as UM171 [79, 80]. Indeed, a trend toward better treatment outcomes along with the development of cell ex vivo culture system was emerged according to our cumulative meta-analysis (Fig. 5). But more clinic trials should be completed to prove the advantage of expansion methods.
Table 3
Expanded and infused cell dose.

| Study          | TNC EF medium and range | CD34+ EF medium and range | Infused manipulated TNC | Infused manipulated CD34+ | Infused unmanipulated TNC | Infused unmanipulated CD34+ | Infused total TNC | Infused total CD34+ |
|----------------|-------------------------|---------------------------|-------------------------|----------------------------|---------------------------|----------------------------|------------------|-------------------|
| 2002 Shpall    | 56(1.03-278)            | 4.0(0.1-20.0)             | nr                      | nr                         | nr                        | nr                        | 0.99×10^7/Kg     | 1.04×10^5/Kg      |
| 2007 Lima      | 26(0.44-275)            | 2.2(0-18)                 | nr                      | nr                         | nr                        | nr                        | 3.6×10^7/Kg      | 1.6×10^5/Kg       |
| 2008 Lima(BMT) | 161(2-620)              | 2.26                      | nr                      | nr                         | nr                        | nr                        | 1.8×10^7/Kg      | 1.5×10^5/Kg       |
| 2008 Lima(Blood) | 23(0.44-275)           | 2.3(0-957)                | nr                      | nr                         | nr                        | nr                        | 3.5×10^7/Kg      | 1.8×10^5/Kg       |
| 2010 Delaney   | 645(192-2161)           | 140(36-688)               | 4.6×10^7/Kg             | 60.3×10^5/Kg               | 3.3×10^7/Kg               | 2.4×10^5/Kg               | 7.9×10^7/Kg      | 62.7×10^5/Kg      |
| 2012 Lima      | 12.2(1.0-29.8)          | 30.1(0-137.8)             | 5.84×10^7/Kg            | 9.5×10^5/Kg                | 2.5×10^7/Kg               | 8.6×10^5/Kg               | 8.34×10^7/Kg     | 18.1×10^5/Kg      |
| 2013 Delaney   | nr                      | nr                        | nr                      | nr                         | nr                        | nr                        | nr               | nr                |
| 2014 Horwitz   | 486(171-643)            | 72(16-186)                | 3.1×10^7/Kg             | 35×10^5/Kg                 | 2.6×10^7/Kg               | 0.7×10^5/Kg               | 5.1×10^7/Kg      | 35.7×10^5/Kg      |
| 2015 Forcade   | nr                      | nr                        | nr                      | nr                         | nr                        | nr                        | nr               | nr                |
| 2016 Wagner    | 854(168-2121)           | 330(67-848)               | 5×10^7/Kg               | 175×10^5/Kg                | 2×10^7/Kg                 | 4×10^5/Kg                | 7×10^7/Kg        | 182×10^5/Kg       |
| 2017 Anand     | nr                      | nr                        | 1.5×10^7/Kg             | nr                         | 2.5×10^7/Kg               | nr                        | 4×10^7/Kg        | nr                |
| 2018 Stiff     | 401.5(0-764)            | 90(6-398)                 | nr                      | nr                         | nr                        | nr                        | 3.06×10^7/Kg     | 1.41×10^5/Kg      |
| 2018 Horwitz   | 1.7                     | 33                        | 4.9×10^7/Kg             | 63×10^5/Kg                 | -                         | -                         | 4.9×10^7/Kg      | 63×10^5/Kg        |
| 2013 Brunstein | -                       | -                         | 1.5×10^7/Kg             | 3.2×10^5/Kg                | 2.5×10^7/Kg               | 3.4×10^5/Kg               | 4×10^7/Kg        | 7.6×10^5/Kg       |
| 2015 Popat     | -                       | -                         | 1.75×10^7/7/Kg          | 0.92×10^5/5/Kg             | 2.59×10^7/7/Kg            | 1.15×10^5/5/Kg            | 4.26×10^7/7/Kg   | 2.37×10^5/Kg      |
| 2013          | -                       | -                         | 1.8×10^7                | 0.74×10                   | 1.7×10^7                  | 0.56×10                  | 3.5×10^7         | 2.37×10           |
Discussion

As mentioned in mechanism, the molecules that acted on signal pathways of homing ability could also activate signal pathways of cell proliferation. Whereas this could not answer the question why the hematopoietic recovery was not accelerated among patients in expansion group, though they received even much more UCB cell dose than patients in incubation group. So a problem was brought into consideration, were the stem cells still "stem cells" after expansion? Sean J. Morrison et al [66] thought the goals of stem cells ex vivo expansion was to preserve the self-renewal ability while inhibit the differentiation tendency. Roberto et al [67] further indicated that in spite of being immunophenotypically similar, freshly isolated stem cells and ex vivo expanded stem cells showed significant differences both in functional and genetic terms. As a result, the long-term hematopoietic capacity of expanded stem cells may be impaired. As was known, the self-renewal, proliferation, differentiation, apoptosis, and bone marrow homing of stem cells were complex physiological processes that were hard to control by artificial regulation. Even the kind and dose of cytokines that should be used in different stem cell growth stage are diverse [68]. Given the fact that the most of the mechanisms behind stem cell proliferation remains unclear, it is hard to expand stem cells without losing their long-term hematopoietic capacity. Importantly, this have been confirmed in our included studies. Even the cell doses of expanded units were much higher than unmanipulated units, the predominance of donor cell engraftment were derived from unmanipulated UCB units rather than expanded units [17, 18]. On the contrary, two incubation studies reported the predominance of long-term engraftments derived from manipulated UCB units [20, 23]. The third one had an opposite result but it was originally proven to be useless in accelerating hematopoietic recovery [19].

Bone marrow niches are vital to stem cells in hematopoietic transplantation for saving them from depletion while protecting the host from over-exuberant stem-cell proliferation [69, 70]. But not all of the infused donor stem cells could engraft into host bone marrow niche [71]. To this end, it is important to enhance the homing ability of infused stem cells. However, no better treatment effects were observed by injecting donor cells into recipient’s BM niche directly [72–74]. Maybe it doesn’t work unless the donor cells are “volunteered” rather than “forced” to go inside the niche. For example, activation of SDF-1α-CXCR4 axis could contribute to donor cells’ volunteering [75–78]. In our included studies, homing ability of stem cells could be enhanced after a short-time incubation and the hematopoietic recovery was accelerated after transplantation. Treatment effects were almost the same compared to expansion studies even with much lower infused cell doses.

Conclusion

It is widely considered that the stem cell dose is vital for hematopoietic recovery during UCBT. As a result, expanding stem cell could be the first choice. But according to our analysis, expansion methods showed no significant improvement in treatment outcomes comparing to incubation methods, most possibly because the stem cells lost long-term hematopoietic capacity after ex vivo culture. In the meantime,
expansion methods still took more resources, such as time and money, than incubation way. And they were also accompanied by the risks of contamination and culture failure.

It is believed that the goal of expanding stem cells while preserving their hematopoietic reconstitution ability can be achieved. Because more effective new molecules will be used in stem cell expansion manipulation with the further research of stem cells’ physiological regulatory mechanisms and continuous optimization of in vitro culture system. However, there is still a long way to go.

**Declarations**

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

**Competing interests**

The authors declare that they have no competing interests.

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**Authors' contributions**

Yang-Yang Lei, Shan-Hong Chang, and Wen-Ming Hua searched the relevant literature and clinical trials. Yang-Yang Lei, Lei Deng, Lu Wang, and Bin Rong collected and analyzed the data. Yang-Yang Lei, Ang Zhang, Mei Guo, Hui-Sheng Ai and Kai-Xun Hu were co-contributor in writing the manuscript. All authors read and approved the final manuscript.

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Figure 1

Flow of the search strategy.
Figure 2

A and B were forest plots of neutrophil recovery time in expansion group and incubation group, respectively. C and D were forest plots of platelet recovery time in expansion group and incubation group, respectively.
Figure 3

Forest plots of 100-day survival in expansion (A) and incubation (B) group.

Figure 4

Intrinsic and extrinsic signals that involved in umbilical cord blood stem cell manipulation procedures.
A

| Study ID | ES (95% CI)       |
|----------|-------------------|
| 2002 Shpall | 29.50 (18.98, 40.02) |
| 2007 Lima   | 23.74 (16.70, 30.78) |
| 2008 Lima   | 25.09 (18.61, 31.57) |
| 2008 Lima   | 21.77 (15.50, 28.04) |
| 2010 Delaney | 21.03 (15.58, 26.47) |
| 2012 Lima   | 19.54 (15.11, 23.97) |
| 2013 Delaney | 19.14 (15.44, 22.84) |
| 2014 Horwitz | 18.59 (15.18, 22.00) |
| 2016 Wagner  | 18.18 (15.26, 21.10) |
| 2017 Anand  | 17.60 (14.67, 20.53) |
| 2018 Stiff  | 18.13 (15.10, 21.15) |
| 2018 Horwitz | 17.77 (14.02, 21.51) |

B

| Study ID | ES (95% CI)       |
|----------|-------------------|
| 2002 Shpall | 91.75 (56.90, 126.60) |
| 2007 Lima   | 63.28 (13.92, 112.64) |
| 2008 Lima   | 59.41 (32.66, 86.15)  |
| 2008 Lima   | 43.93 (33.98, 53.88)  |
| 2012 Lima   | 41.65 (35.69, 47.61)  |
| 2013 Delaney | 44.35 (38.04, 50.66)  |
| 2014 Horwitz | 43.15 (37.38, 48.92)  |
Figure 5

Cumulative meta-analysis of neutrophil (A) and platelet (B) recovery time of expansion group.