Feasibility and safety of autotransplants with noncryopreserved marrow or peripheral blood stem cells: a systematic review

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The objective of this systematic review is to examine the feasibility and safety of autologous noncryopreserved stem-cell transplants. This technique avoids the cost of establishing and maintaining a cryopreservation facility and may be of value for transplant centers in regions with limited economic resources. The primary outcome was the graft failure rate. In addition, a detailed description of the high-dose therapy regimens employed was undertaken. Secondary outcomes were transplant-related mortality and neutrophil and platelet engraftments times. Sixteen well-conducted nonrandomized studies met the eligibility criteria. Only two cases of graft failure (0.36%) occurred among 560 assessable patients receiving high-dose therapy and autotransplant for non-Hodgkin’s lymphoma, Hodgkin’s lymphoma, multiple myeloma, germ-cell tumors and acute leukemias. The most traditional high-dose schedules were used, although often modified to shorter regimens. High-dose melphalan appeared especially useful given its short half-life and was used to treat multiple myeloma by most groups. Secondary outcomes were comparable to those reported in the most relevant studies addressing standard (cryopreserved) autotransplant. According to this study, the use of autologous noncryopreserved hematopoietic progenitors to support patients undergoing high-dose therapy is feasible and safe.

Key words: hematopoietic, liquid storage, non-cryopreserved, nonfrozen

Introduction

High-dose therapy and autotransplant are the standard of care in the treatment of many malignancies. Randomized trials show improved overall survival for autotransplants over standard chemotherapy in patients with relapsed intermediate or high-grade non-Hodgkin’s lymphoma (NHL) [1, 2] and multiple myeloma [3, 4]. For other diseases, single-arm studies show encouraging survival outcomes while other randomized trials demonstrate significant delays in disease progression. This is the case for primary refractory NHL and Hodgkin’s lymphoma (HL) [5–9], relapsed HL [7, 10], transformed lymphoma [11–13], mantle cell lymphoma in first remission [14] and relapsed or primary refractory germ-cell tumors [15–20]. Promising results have been obtained in other malignancies such as acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), amyloidosis and other entities [21, 22].

As most high-dose therapy schedules were designed to deliver cytotoxic drugs over a number of days [e.g. 6 days for BEAM (BCNU, etoposide, cytarabine and melphalan), CBV (cyclophosphamide, BCNU and etoposide) or ICE (ifosfamide, carboplatin and etoposide)] [23–26], freezing of the harvest product was traditionally carried out to maintain cell viability until stem-cell reinfusion. The liquid storage of harvested stem cells, either at room temperature or in standard blood refrigerators, is an alternative to cryopreservation. This technique may be of value in two scenarios. Most importantly, it could be used—and is being used—by some medical institutions from areas with limited economic resources that, while having an adequate infrastructure to treat malignant conditions such as acute leukemia, do not have the funding to support cryopreservation facilities. This is the case for many developing countries that invest in the treatment of acute leukemia but do not have the infrastructure to support autotransplantation. Noncryopreserved liquid storage could be a way to maintain the cell viability of the harvest product until reinfusion. Second, this technique could be of interest to centers that require expansion of their autotransplant treatment capacity but do not have the laboratory space or the resources to establish a cryopreservation facility. Noncryopreserved liquid storage could be an alternative strategy for the preservation of harvested cells to support the additional transplantation needs of these centers.

This technique has received little attention even though some groups from different countries have used it since the earliest period of autotransplant development. Preclinical data supporting the use of noncryopreserved stem cells are available since 1957. Studies carried out in mice reported successful rescue from lethal total body irradiation (TBI) with reinfusion of marrow cells stored at 25°C for 11 days [27]. Subsequent in vitro and clinical studies demonstrated that human bone marrow (BM) cells could be preserved for 48 h to 9 days in the liquid state without significant loss of granulocytic/macrophage-committed progenitor cells, providing hematological reconstitution to patients receiving myeloablative therapy [28–32]. This technique may be of value in two scenarios. Most importantly, it could be used—and is being used—by some medical institutions from areas with limited economic resources that, while having an adequate infrastructure to treat malignant conditions such as acute leukemia, do not have the funding...

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to establish a cryopreservation facility associated with a traditional blood and marrow transplant program [32, 33]. Secondly, it could be implemented as a first step in establishing a transplant program that eventually will have cryopreservation capability.

The purpose of this study is to examine the accumulating evidence of the feasibility and safety of noncryopreserved autotransplants. This is, to our knowledge, the first systematic review on the subject.

methods

literature search

The literature that has been published since 1965 in any language on the subject of noncryopreserved autotransplants was reviewed. The search incorporated the use of Medline, EMBASE, the Cochrane Database of Systematic Reviews, abstracts from the American Society of Hematology and the American Society of Clinical Oncology (ASCO) meetings, as well as http://www.Clinicaltrials.gov/ and http://www.google.com. The reference list of every retrieved study was consulted. Main keywords were ‘non-cryopreserved’, ‘noncryopreserved’, ‘non-frozen’, ‘nonfrozen’ and ‘liquid storage’. The keyword ‘cryopreservation’ was combined with ‘bone marrow’, ‘peripheral blood’, ‘stem cell’ or the MeSH terminologies ‘Transplantation, Autologous’ and ‘Hematopoietic Stem Cell Transplantation’ while the Boolean operator NOT was introduced to exclude the terms ‘ovary’, ‘ovarian’, ‘cord’ and ‘allogeneic’.

article selection criteria

A first screening was carried out to select all articles describing patients treated with high-dose myeloablative therapy for malignancy [34] then rescued with noncryopreserved hematopoietic progenitors from the BM or the peripheral blood (PB), in the latter case collected by leukapheresis. Studies evaluating rescue with growth factor-primed whole blood were not included. The second screening focused on articles reporting all of the following: diagnosis, age, high-dose regimen used (including drug dosage), number of harvested progenitors, duration and condition of storage, engraftment times for neutrophils and platelets, transplant-related mortality (TRM) and graft failure rate. All study designs from any source (peer-reviewed journal, non-peer-reviewed source or scientific meeting abstract) were considered with the condition that completeness of the information as defined previously was a requirement. Only the most complete or up-to-date publications were included for studies with overlapping patient populations.

outcomes

Primary objectives of this review are as follows: (i) to establish the safety of noncryopreserved hematopoietic stem cells to provide hematopoietic rescue after high-dose therapy and (ii) to provide a description of those high-dose regimens available to be used in the context of noncryopreserved autotransplants for the diseases gathering most autograft prescriptions such as HL, NHL, multiple myeloma, germ-cell tumors and acute leukemias.

The cumulative graft failure rate was chosen as the main outcome measure because this variable has a direct relationship with the capacity of stem cells to reconstitute the hematopoietic system and is independent of the source of stem cell used. In addition to progenitor viability, other factors such as an inadequate number of reinfused progenitors, viral infections, drug toxicity and impaired marrow stromal function from prior chemotherapy or radiation can lead to a graft failure [35]. As the definition of graft failure was expected to be heterogeneous among studies—especially regarding time periods used as a threshold—we anticipated potential interference with the competing variable, TRM for cases of patients dying in aplasia. To avoid interference, we defined graft failure as the lack of neutrophil recovery to \( >0.5 \times 10^9/l \) by transplant day +28 and/or transfusion dependence to maintain platelet numbers \( >20 \times 10^9/l \) after day +100. All deaths in aplasia in patients fulfilling our criteria for graft failure were considered a case of graft failure, while deaths in aplasia occurring before these time limits were considered transplant related.

Secondary outcomes were established as follows: neutrophil engraftment was defined as the number of days to attain a neutrophil count \( >0.5 \times 10^9/l \) from the day of reinfusion of stem cells (day 0); platelet engraftment was defined as the number of days necessary to attain an unsupported platelet count \( >20 \times 10^9/l \) from day 0 and TRM was defined as the probability of dying without disease recurrence, accounting for all deaths related to the transplant procedure in a cumulative fashion, regardless of the number of days after transplant they occurred.

data synthesis

Data from this systematic review were synthesized in a descriptive or nonquantitative manner. This included the tabulation of study characteristics and outcomes. The rationale for taking this approach included the existence of heterogeneity among the studies that met the inclusion criteria. These include differences in primary diagnoses, disease status at transplantation, high-dose therapy regimen, stem-cell source and definitions of end points. Differences were also noted in the design of studies. Despite these differences, there was very good consistency in the proportion of cases with graft failure among the studies, obviating the need for the calculation of an overall average effect.

results

search results and selection process

The Medline search with the keyword non-cryopreserved retrieved most papers \( (N = 37) \). One article was obtained from EMBASE and three from http://www.google.com. Six abstracts were obtained from http://www.bloodjournal.org (ASH meetings 2002–2004) and three from http://www.ascos.org (ASCO meetings 1995–2005). One study was found by checking additional publications of the authors of selected studies, resulting in a total of 51 articles screened.

Reasons to exclude articles included incompleteness of the data for graft failure, time to neutrophil and platelet engraftment and description of the high-dose regimen impeding its categorization into myeloablative or nonmyeloablative. Two articles with complete data were excluded; one of them had major methodological flaws—detected by an external audit carried out by the publishing journal—that led to the retraction of its publication. The second article, though not retracted by the publishing journal, had similar authorship, institutional affiliation and was contemporaneous with the retracted study. None of the excluded articles implicitly or explicitly reported cases of graft failure.

Sixteen articles met all the eligibility criteria and were included in the final analysis (Table 1). All papers except one phase II multicenter trial were retrospective reviews of case series, either from single or multiple centers (Table 2).

stem-cell sources, dose and storage conditions

Ten papers addressing noncryopreserved BM transplant were published between 1984 and 2002. Experience with
noncryopreserved peripheral blood progenitor cell transplant (PBPC) was published between 1995 and 2006 in six papers. BM was obtained by multiple aspirations from the posterior and anterior iliac crests with BM harvest needles. In some cases, BM was also collected from the sternum. In all but one paper, BM was harvested without previous ‘priming’ with hematopoietic growth factors. One group used priming of BM with hematopoietic growth factors on four of 27 patients before harvest [36]. Median mononuclear cell (MNC) yield varied among different reports, ranging between $1.4 \times 10^8$ and $3.44 \times 10^8$ MNC/kg (absolute range $0.4–11.9 \times 10^8$ MNC/kg). In nine of 10 studies, the harvest product was kept at 4°C. Other storage conditions reported were room temperature and refrigeration at 1°C (Table 3). The total storage period ranged between 8 and 72 h. BM was reinfused unprocessed by eight groups, passed through incremental filters of standard marrow collecting kits in one case and centrifuged and depleted of the fatty supernatant in an additional case.

PB progenitor cells were stored at 4°C for 24 h to 6 days before reinfusion (Table 4). Median concentrations of CD34+ progenitors ranged between $3 \times 10^6$ and $5.5 \times 10^6$ CD34+/kg (absolute range $0.16–27.8 \times 10^6$ CD34+/kg).

**primary outcomes**

*high-dose therapy regimens.* High-dose therapy regimens used by the different groups were heterogeneous. Drugs were used as single agent, multiple combinations or associated with TBI. Compounds included nitrogen mustards [melphalan and cyclophosphamide (CTX)], alkyl sulfonates (busulfan), nitrosoureas (BCNU), aziridines (thiotepa), epipodophyllotoxins (etoposide), platinum complexes (carboplatin), pyrimidine analogues (cytarabine) and microtubule agents (vinblastine). Single fraction TBI was used at doses of 950–1200 cGy in combination with CTX 120 mg/kg [32]. Multiple fraction TBI was used in combination with melphalan 3 mg/kg at a total dose of 1050 cGy in fractions of 350 cGy delivered over 36 h [37]. Autografts were reinfused to patients 24–48 h after the completion of chemotherapy. The only exception was melphalan monotherapy, for which reinfusion was carried out after 8 h by one group [38]. Table 5 summarizes the high-dose therapy schedules used for specific diseases.

*multiple myeloma*

Melphalan becomes undetectable in plasma and urine 1 and 6 h, respectively, after an i.v. infusion of a high dose.
Noncryopreserved stem cells were reinfused as early as 8 h after high-dose melphalan and full and rapid hematopoietic reconstitution was attained [38]. At least eight groups used the agent in the setting of noncryopreserved autotransplant. Three groups treated multiple myeloma patients [29, 32, 33], two groups treated solid tumors [38, 39], two groups treated NHL [37, 40] and one additional group treated HL patients [41] (Tables 2 and 3). Doses ranged between 140 and 220 mg/m². Stem cells were reinfused 8–24 h after the i.v. administration of melphalan. There were no cases of graft failure reported among these patients.

**lymphoma**

Various schedules were employed to treat lymphoma patients (Table 5). Six groups used CBV variants in 152 patients. Drugs were delivered over 3 days (Table 5). The source of stem cells was BM in five studies [28, 36, 40, 42, 43] and PBPC in one [32]. Successful engraftment was attained in all cases treated with CBV. Taylor et al. [44] treated 17 patients with advanced HL with melphalan 3 mg/kg (>15 min) and etoposide 1600 mg/m² infused over 20 h followed by noncryopreserved BM autograft reinfused within 56 h after harvest (including a 24-h drug clearance). There were no treatment-related deaths and complete hematological reconstitution was universally achieved. Papadimitriou et al. [29] also used melphalan 140 mg/m² + etoposide 1500 mg/m² followed in this case by noncryopreserved PBPC support to treat one HL and two NHL patients. The harvest product was reinfused 24–48 h after the completion of chemotherapy. Neither graft failure nor TRM was observed among them. Ager et al. [45] treated seven NHL, two HL patients and one case of renal amyloidosis with etoposide 2 g/m² infused over 4 h on day –3 and melphalan 140 mg/m² on day –1, with the addition of BCNU 300–450 mg/m² as a 3-h infusion on day –2. No cases of TRM or graft failure were registered.

**acute leukemia**

Most series of noncryopreserved autotransplants for acute leukemia used multiagent high-dose chemotherapy without TBI. Köppler et al. [43] published a case series of 21 AML patients either in first or subsequent complete remission. The high-dose regimen was CTX 60 mg/kg 2×, etoposide 700 mg/m² 3× and cytarabine 1 g/m² 6× (every 12 h). Noncryopreserved autologous BM was reinfused within 72 h from harvest. Engraftment was attained in all cases and no treatment-related deaths were reported.

A large series of 101 noncryopreserved autotransplants for high-risk ALL in first complete remission was published as an abstract in 2002 by Holowiecki et al. [46]. Graft failures were not observed. TRM was 3% and all deaths were due to septic infections, one on day +9 and two on day +12 (Professor J. Holowiecki, personal communication). The high-dose regimen included CTX 60 mg/m² on days –3 to –2 + etoposide 800 mg/m² on days –3 to 2 + cytarabine 1 g/m² every 12 h (6×) on days –3 to –1. Tables 2, 3 and 4 show other high-dose combinations that have been used for acute leukemia in this setting.

**germ-cell tumors**

Papadimitriou et al. [29] treated four patients with CTX 60 mg/kg, carboplatin 900 mg/m² and etoposide 2500 mg/m² on day –1 or –2 (Table 5). PBPC were reinfused 24–60 h after chemotherapy. Engraftment occurred uneventfully in all patients and no transplant-related deaths were reported (Table 4). Other groups treated a variety of tumors using regimens that could be suitable for the treatment of testicular cancer, too. Köppler et al. [43] treated 14 patients with solid tumors (not otherwise specified) with CTX 60 mg/kg 2×.
<table>
<thead>
<tr>
<th>Table 3. Patients’ characteristics and results of noncryopreserved autologous bone marrow transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of</strong></td>
</tr>
<tr>
<td>patients</td>
</tr>
<tr>
<td>Kingston et al. [38]</td>
</tr>
<tr>
<td>Carella et al. [42]</td>
</tr>
<tr>
<td>Russell et al. [41]</td>
</tr>
<tr>
<td>Ahmed [28]</td>
</tr>
<tr>
<td>Carey et al. [37]</td>
</tr>
<tr>
<td>Köppler et al. [43]</td>
</tr>
<tr>
<td>Sierra et al. [40]</td>
</tr>
<tr>
<td>Taylor et al. [44]</td>
</tr>
<tr>
<td>Holowiecki et al. [36]</td>
</tr>
<tr>
<td>Holowiecki et al. [46]</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*aSee Table 5 for details of dosages and schedule.

MNC, mononuclear cells; CSF, colony-stimulating factor; TRM, transplant-related mortality; GF, graft failure; NB, neuroblastoma; ET, Ewing’s tumor; WT, Wilm’s tumor; RMS, rhabdomyosarcoma; OS, osteosarcoma; MEL, melphalan; RT, room temperature; BCNU, carmustine; HL, Hodgkin’s lymphoma; NHL, non-Hodgkin’s lymphoma; CBV, cyclophosphamide, BCNU and etoposide; WBC, white blood cell; CTX, cyclophosphamide; TBI, total body irradiation; Thio, ThioTEA; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CAV, combination chemotherapy with cyclophosphamide, doxorubicin and vincristine; CBDCA, carboplatin; VP16, etoposide; Vbl, vinblastine; CR1, first complete remission; CR2, second complete remission.
### Table 4. Patients' characteristics and results of noncryopreserved autologous peripheral blood stem-cell transplantation

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Age range</th>
<th>Diagnosis</th>
<th>High-dose therapy*</th>
<th>No. of reinfused progenitors CD34+ cells x10⁶ (median and range)</th>
<th>Storage conditions and duration</th>
<th>CSF post-stem-cell infusion</th>
<th>Neutrophils 20.5 x 10⁹/l (median and range)</th>
<th>Platelets &gt;20 x 10⁹/l (median and range)</th>
<th>TRM patient # (%)</th>
<th>GF patient # (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ager et al. [45]</td>
<td>10</td>
<td>16–53</td>
<td>NHL (7), HL (2), Amy (1)</td>
<td>MEL/VP16/BCNU 5.5 (2.1–13)</td>
<td>4°C, 4 days</td>
<td>N</td>
<td>12.5 (10–25)</td>
<td>13.5 (11–44)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jones et al. [39]</td>
<td>5</td>
<td>4–14</td>
<td>RMS (2), NB (1), PNET (1), MB (1)</td>
<td>MEL 200 10 (8.1–19.4)</td>
<td>4°C, 48–72 h</td>
<td>N</td>
<td>11 (10–16)</td>
<td>14 (12–31)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Papadimitriou et al. [29]</td>
<td>72</td>
<td>8–69</td>
<td>MM (33), NHL (2), HL (1), AML (1), Amy (1), SCLC (3), OC (26), TC (4), other (1)</td>
<td>MEL 140–180, MEL/VP16, CBDCA/VP16/CTX, other</td>
<td>4°C, 24–60 h</td>
<td>Y</td>
<td>9 (6–16)</td>
<td>5 (0–89) (&gt;25 x 10⁹/l)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ruiz Argüelles et al. [33]</td>
<td>46</td>
<td>9–67</td>
<td>AML (13), ALL (9), CML (4), MM (6), HL (7), NHL (3), MBC (4)</td>
<td>MEL 200 4.68</td>
<td>4°C, 24–72 h</td>
<td>N</td>
<td>14 (0–86)</td>
<td>25 (0–102)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Cuellar-Ambrosi et al. [32]</td>
<td>47</td>
<td>12–67</td>
<td>NHL (21), MM (10), ALL (3), AML (3), LCH (1), BC (1)</td>
<td>CBV, CTX/TBI, MEL200, MEL/CBDCA Group 1, 1.36 (0–6.32)</td>
<td>4°C, 6 days</td>
<td>Y</td>
<td>Group 1, 11 (9–15); Group 2, 13 (10–17); Group 3, 11 (10–16)</td>
<td>6 (12.7)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Mabed and Al-Kgodary [47]</td>
<td>32</td>
<td>17–55</td>
<td>NHL</td>
<td>CBDCA/VP16/CTX Group 5, mean 12.7 MNC x 10⁹/kg</td>
<td>&gt;3</td>
<td>4°C, 72 h</td>
<td>N</td>
<td>12 (8–17)</td>
<td>14 (7–19) (3.97)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>212</td>
<td></td>
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<td></td>
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<td></td>
<td>0</td>
</tr>
</tbody>
</table>

*See Table 5 for details of dosages and schedule.

CSF, colony-stimulating factor; TRM, transplant-related mortality; GF, graft failure; NHL, non-Hodgkin’s lymphoma; HL, Hodgkin’s lymphoma; Amy, amyloidosis; MEL, melphalan; VP16, etoposide; BCNU, carmustine; RMS, rhabdomyosarcoma; NB, neuroblastoma; PNET, primary neuroendocrine tumor; MB, medulloblastoma; MNC, mononuclear cell; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MM, multiple myeloma; SCLC, small-cell lung cancer; OC, ovarian cancer; TC, testicular cancer; CBDCA, carboplatin; CTX, cyclophosphamide; ALL, acute lymphoblastic leukemia; MBC, metastatic breast cancer; LCH, Langerhans cell histiocytosis; BC, breast cancer; CBV, cyclophosphamide, BCNU and etoposide; TBI, total body irradiation; CFU-GM, colony-forming unit-granulocyte/macrophage.
### Table 5. High-dose therapy schedules employed for specific diseases from trials evaluating hematopoietic rescue with noncryopreserved stem cells

<table>
<thead>
<tr>
<th>Disease</th>
<th>HDT combination</th>
<th>Dosage and schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td>CBV</td>
<td>CTX 4800–5000 g/m² + etoposide 1500–2000 mg/m² + BCNU 400–600 mg/m² [28]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTX 60 mg/kg on days −3,−2 + etoposide 700–800 mg/m² on days −3,−2,−1 + BCNU 400 mg/m² on day −3 [36, 51]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTX 120 mg/kg + etoposide 400 mg/m² + BCNU 300 mg/m² [32]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTX 5 g/m² + etoposide 400 mg/m² + BCNU 600 mg/m² [40, 42]</td>
</tr>
<tr>
<td></td>
<td>MEL 200</td>
<td>Melphalan 200 mg/m² [33]</td>
</tr>
<tr>
<td></td>
<td>MEL/VP16</td>
<td>Melphalan 140 mg/m² + etoposide 1500 mg/m² [29]</td>
</tr>
<tr>
<td></td>
<td>MEL/VP16/BCNU</td>
<td>Etoposide 2000 mg/m² as a 4 h infusion −3, BCNU 300–450 mg/m² &gt;3 h on day −2, melphalan 140 mg/m² i.v. bolus on day −1 [45]</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>MEL 180–200</td>
<td>Melphalan 200 mg/m² [32, 33]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Melphalan 180 mg/m² [29]</td>
</tr>
<tr>
<td>Testicular germ-cell tumors</td>
<td>CBDA/VP16/CTX</td>
<td>Carboplatin 900 mg/m² + etoposide 2500 mg/m² + CTX 60 mg/kg [29]</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>CTX/TBI</td>
<td>CTX 120 mg/kg + unfractioned TBI 1200 cGy [32]</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>CAV/TBI</td>
<td>Cytarabine 1 g/m² every 12 h 6× + etoposide 700 mg/m² 3× + CTX 60 mg/kg 2× [43]</td>
</tr>
<tr>
<td></td>
<td>MEL/TBI</td>
<td>Melphalan 3 mg/kg + fractioned TBI (1050 cGy) [37]</td>
</tr>
<tr>
<td></td>
<td>CTX/2 TBI</td>
<td>CTX 120 mg/kg + unfractioned TBI 1200 cGy [32]</td>
</tr>
<tr>
<td></td>
<td>CAV</td>
<td>Cytarabine 1 g/m² every 12 h (6×) on days −3, −2 and −1 + etoposide 800 mg/m²² on days −3 and −2 + CTX 60 mg/m²² days −3 and −2 [46]</td>
</tr>
</tbody>
</table>

HDT, high dose therapy; CBV, cyclophosphamide; BCNU and etoposide; CTX, cyclophosphamide; BCNU, carmustine; MEL, melphalan; VP16, etoposide; CBDA, carboplatin; TBI, total body irradiation; CAV, combination chemotherapy with cyclophosphamide, doxorubicin and vincristine.

etoposide 700 mg/m² 3× and carboplatin 1200 mg/m² 1×.

Noncryopreserved BM was reinfused within 72 h after harvest (Table 5). In addition, Mabed and Al-Kgoday [47] treated 32 NHL patients with CTX 60 mg/kg/day for 2 days, etoposide 15 mg/kg/day for 2 days and carboplatin 400 mg/m³/day for 2 days with grafts reinfused 72 h after harvest, leading to full hematopoietic recovery in all patients (Table 4).

graft failure. No cases of graft failure were seen in 14 of 16 studies. One group reported a patient dying in aplasia on transplant day +40 [42]. This patient received 0.4 × 10⁹ MNC/kg, the lowest dose among all 404 patients from 10 studies evaluating noncryopreserved BM autotransplants. A second study reported the case of a patient dying from a nonspecified cause before reaching an unsupported platelet count >50 × 10⁹/l (threshold used by this group to define platelet engraftment).

As seven patients died early before engraftment [33, 36, 38, 40, 46], two cases of graft failure occurred among 609 evaluable procedures. Fifty-six (9.2%) patients from five studies, however, received nonmyeloablative doses of melphalan of 140 mg/m², 160 mg/m² or 3 mg/kg [29, 37, 38, 40, 41]. As the use of nonmyeloablative regimens can lead to an underestimation of the graft failure rate, these patients were excluded from the final analysis. Consequently, two of 553 patients (0.36%) failed to engraft.

**secondary outcomes**

**neutrophil and platelet engraftment with noncryopreserved BM**. Among 10 studies in which autologous noncryopreserved BM was used, seven defined neutrophil engraftment as the median number of days to a neutrophil count ≥0.5 × 10⁹/l from transplant day 0. Medians ranged between 10 and 27 days and absolute limits were five and 103 days (Table 3). Three other studies reported neutrophil engraftment as the time to achieve white blood cell (WBC) ≥1 × 10⁹/l. Outcomes are shown in Table 3.

Platelet engraftment data were defined as the median time to reach an unsupported count of >20 × 10⁹/l by four groups, with medians ranging between 18 and 28 days (absolute 5–300 days). Four additional papers used a nonsupported count of ≥50 × 10⁹/l as threshold and two groups defined platelet engraftment as the number of days with transfusion requirement (Table 3).

Retrospective comparisons of cryopreserved versus noncryopreserved BM rescue are available. Sierra et al. [40] analyzed a cohort of 94 NHL patients rescued with cryopreserved and 38 with noncryopreserved BM autografts. No statistically significant differences were found in time to a granulocyte count >0.5 × 10⁹/l (median 20 and 22 days for noncryopreserved and cryopreserved, respectively, P = 0.47) or a platelet count >20 × 10⁹/l (median 28 and 27 days, for noncryopreserved and cryopreserved grafts, respectively, P = 0.54). TRM was 13% and 22%, for the noncryopreserved and the cryopreserved groups, respectively (P = 0.36). Ahmed et al. [28] published a similar study. Thirty-eight patients had BM stored at 4°C for a mean of 3 days (range 2–5 days) and 15 patients had cryopreserved marrow stored for an average of 56 days. The median nucleated cell count was 3.0 × 10⁹/kg for the noncryopreserved group and 2.5 × 10⁹/kg for the cryopreserved one. Time to WBC recovery to >1 × 10⁹/l was 17 days for the
noncryopreserved group and 23 days for the cryopreserved one. Time to platelet recovery to $>20 \times 10^9/l$ was 24 and 51 days for the noncryopreserved and cryopreserved groups, respectively.

**Neutrophil and platelet engraftment using noncryopreserved PBPC.** Results of neutrophil engraftment to $20.5 \times 10^9/l$ are available from six studies. Median times to neutrophil recovery ranged between 9 and 14 days, with absolute limits of 0 and 86 days (Table 4).

Platelet engraftment was presented as the median number of days to attain an unsupported number of $>20 \times 10^9/l$ by five groups. Medians ranged between 13.5 and 25 days, with absolute limits from 0 to 102 days (Table 4). One group used a threshold of $25 \times 10^9$ instead (median 5 days, range 0–89) [29].

**Transplant-related mortality.** Twenty-two transplant-related deaths occurred among 616 procedures (3.65%). There were no treatment-related deaths in eight studies, while eight groups reported two toxic deaths (multiorgan dysfunction) and four infections (two gram-negative sepsis, one mucormycosis and one pulmonary tuberculosis). Sierra et al. [40] had a TRM of 13% caused by one case of each of the following: infection, liver veno-occlusive disease, heart toxicity, interstitial pneumonitis and one unspecified case.

Data on late transplant complications among 114 patients with NHL (62) and HL (52) autografted between 1984 and 1995 were reported by Taylor et al. [48]. Patients received melphalan/TBI (26), melphalan/etoposide (66) or melphalan alone (50). Median follow-up was 62 months. Two new hematological malignancies occurred, both in HL patients (one chronic myeloid leukemia (CML) and one AML, 18 and 20 months after the autograft, respectively).

**Discussion**

Concerns regarding adequate hematopoietic reconstitution may arise whenever noncryopreserved autotransplants are considered. The results of the present study, however, indicate otherwise. Hematopoietic reconstitution reported by all groups was almost universal; only two cases of graft failure were documented among 560 assessable patients receiving myeloablative therapy and autologous noncryopreserved stem cells. Moreover, one of the graft failure cases represented a patient given a likely inadequate dose of BM cells ($0.4 \times 10^8$ MNCs/kg) [42].

Potential biases underestimating graft failure rates were taken into account during the design of the present review. For example, a bias could be introduced if nonmyeloablative high-dose therapy was used because of the high likelihood of hematological recovery without the need for stem-cell rescue. To avoid treatment bias, we required all evaluable papers to have full drug dose data. A small proportion of patients from five studies included in the analysis who received nonmyeloablative doses of chemotherapy were excluded from the final analysis of graft failure [29, 37, 38, 40, 41].

A selection bias could be generated during the review process, if papers reporting cases of graft failure were excluded from the final analysis. As shown in Table 1, however, there were no graft failures among the excluded papers addressing the clinical use of noncryopreserved BM or PBPC autotransplant.

Since clinical experience leading to negative results (represented in this case by high rates of graft failure) is less likely to be sent for publication, we acknowledge the potential problem of publication bias. Although a comprehensive literature search that included multiple databases to track all possible research resulting in negative outcomes was carried out, this may not be sufficient to eliminate this bias. Multiple heterogeneities were found among the available studies including disease diversity, multiplicity of high-dose schedules and lack of uniform definitions for the variables we used as outcome measures. This heterogeneity could not be disregarded and hindered a quantitative comparison of outcomes against data from relevant randomized trials that evaluated the standard autologous stem-cell transplantation for specific diseases as control group. Heterogeneity and publication bias are probably the most important limitations of the present review.

Stem cells can be stored without cryopreservation for only a few days; consequently, some of the most widely used high-dose schedules, such as those requiring delivery over 26 days, would, of necessity, be excluded. For this reason, another objective of our review was to provide a detailed description of the intensive therapy regimens used for the most common indications for autotransplant that would be compatible with noncryopreserved autograft support. Patients with HL, NHL, germ-cell tumors and acute leukemia have received shorter versions of commonly used high-dose schedules to avoid long periods of storage of noncryopreserved autografts. Specifically designed regimens have also been employed that can be delivered over no more than 3 days.

In our opinion, the rule ‘the sooner the better’ applies in this context and stem cells should be rein infused within 3–5 days from harvest. Several preclinical studies demonstrated that cell viability assessed by trypan blue test [49], granulocyte–macrophage colony-forming units numbers [31, 49], erythroid burst-forming units, mixed lineage colony-forming units [49], CD34+ cell number and long-term culture-initiating cells [50] are unchanged after 2–3 days of liquid storage at 16°C, while up to 79% of cells are still viable for up to 5 days. Moreover, Ager et al. [45] and Cuellar-Ambrosi et al. [32] have reinfused PB stem cells after 4 and 6 days of unfrozen storage, respectively, obtaining full engraftment in all cases as shown in Table 4.

Autologous transplantation is a supportive method that renders high-dose chemotherapy a well-tolerated treatment option for cancer patients. Unlike allotransplants, the antitumor effect in the case of an autologous transplantation is exerted exclusively by the intensive regimen and not by the reinfused hematopoietic progenitors. The aim of the present review is to analyze the efficacy and safety of noncryopreserved autografts as an alternative supportive method. The heterogeneous baseline characteristics of the patients from the 16 papers hindered the analysis of survival outcomes given that the low number of cases with uniform diagnoses and disease status at transplant do not...
Table 6. Advantages and disadvantages related to the use of noncryopreserved stem cells

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Transplant cost reduction</td>
<td>• Limitation for the use of some traditional high-dose schedules as a result of the limitation of the stem-cell storage period</td>
</tr>
<tr>
<td>• Simplicity of implementation</td>
<td>• Less flexible allocation of resources: it requires a more efficient coordination of stem-cell mobilization, apheresis, administration of the high-dose therapy and the stem-cell reinfusion</td>
</tr>
<tr>
<td>• Expansion of the number of centers offering autotransplants</td>
<td></td>
</tr>
<tr>
<td>• Facilitated implementation of transplantation in a center that will eventually have cryopreservation capability</td>
<td></td>
</tr>
<tr>
<td>• Prevention of dimethyl sulfoxide toxicity</td>
<td></td>
</tr>
<tr>
<td>• Time saving between last induction chemotherapy and high-dose therapy</td>
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</tr>
</tbody>
</table>

allow making any relevant conclusions. We can indicate, however, that in view of the low rate of TRM observed, the fact of supporting patients with noncryopreserved rather than cryopreserved progenitors should not likely impact survival outcomes. Besides this toxicity results, however, an intrinsic effect of the noncryopreserved progenitors on survival cannot be entirely excluded.

Although the use of noncryopreserved stem-cell products have the benefit of avoiding the costs and facilities for cryopreservation, it should be noted that this approach mandate more precise planning of resource allocations as patients must progress to reinfusion soon after collection. This will require an efficient coordination of stem-cell mobilization, apheresis, administration of the high-dose therapy and the stem-cell reinfusion that may be more flexible in centers that use cryopreservation (Table 6).

In conclusion, this systematic review supports the feasibility and safety of noncryopreserved autotransplants. This technique, carried out by institutions and doctors with expertise in hematopoietic transplants, seems to be a safe alternative to the standard procedure and may be especially useful in centers in which cryopreservation of hematopoietic stem cells is not available.

references


