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A haploidentical transplant can be defined as a transplant that uses marrow or stem cells from a relative who is only half matched in HLA. Recently, a new regimen using graft CD3/CD19 depletion with anti-CD3- and anti-CD19-coated microbeads on a CliniMACS device was developed. CD3/CD19 depleted grafts contain not only CD34+ stem cells but also CD34- negative progenitors, natural killer, dendritic and graft-facilitating cells, thereby enhancing engraftment. We report a case description of a child using CD3/CD19 depleted grafts in a haploidentical stem cell transplant setting and a review of the available literature. A 3-year old boy was treated for very early relapse of acute lymphoblastic leukaemia. The myeloablative therapy consisted of weight-adjusted intravenous busulfan, fludarabine, cyclophosphamide, and thymoglobuline. The reduction of CD3 load was 99.9% (i.e. 3.64 log scale); however, due to current CD3 load in the graft (2.7×10^5 CD3+/kg), GVHD prophylaxis was done with cyclosporine A and methotrexate. The only complication after transplantation was CMV reactivation, treated with pre-emptive therapy. Bone marrow biopsy at day +29 revealed normal haematopoiesis and donor chimerism 95%. The patient remains in remission at day +300. We can conclude from our observation and literature review that HLA haploidentical peripheral blood stem cell transplantation with CD3/CD19 depletion may be an effective and safe therapy for children with relapsed leukaemia.

Key words: stem cell transplantation, children, CD3/CD19 depletion, acute lymphoblastic leukaemia.

Haploidentical allogeneic haematopoietic cell transplantation using CD3/CD19 depletion and myeloablative conditioning: a case report and a review of the literature

Haploidentyczne przeszczepienie allogenicznych komórek krwiotwórczych z deplecją CD3/CD19 i kondycjonowaniem mieloablacyjnym: opis przypadku i przegląd piśmiennictwa

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Introduction

From a historical point of view, one of the principles of transplantation is that in an allo-HSCT setting, human leukocyte antigens (HLA) should be the same for the donor and patient. However, only about 25% of patients have an HLA-identical ("matched") sibling donor and about 50-60% have a matched unrelated donor. Still about 15-25% of patients requiring transplantation lack appropriate donors. Current experience indicates that transplantations made from a donor with one or two mismatches in HLA (out of 10 major HLA loci) are feasible and safe for patients, but are associated with a high risk of complications. Nevertheless, for a small percentage of patients, neither a sibling nor unrelated donor is available, so haploidentical transplantation is an important curative opportunity for them.

A haploidentical transplant can be defined as a transplant that uses marrow or stem cells from a relative who is only half matched in HLA. Children inherit half of HLA proteins from their mother, and half from their father. Haploidentical donors share one haplotype (an identical set of HLA antigens from a family member) and are mismatched for at least one antigen on the other haplotype.

A majority of patients requiring allogeneic stem cell transplantation lack a sibling donor. In childhood acute lymphoblastic leukaemia, a donor should be available within a short time, because high-risk patients in first, second or subsequent complete remission should be transplanted within three months. Since virtually every patient has a potentially suitable haploidentical related donor as parents or children, a successful strategy for haploidentical allogeneic haematopoietic cell transplantation (HHCT) would eliminate the "lacking donor" problem. Haploidentical parental donors offer some advantages: donors are available when needed, they are highly motivated, and they are, in contrast to unrelated or cord blood donors, available if in case of rejection or non-engraftment a second donation is necessary or donor lymphocyte infusion (DLI) is requested for immunotherapy to prevent relapse. There are also some disadvantages: initially, trials of HHCT were complicated

Przeszczepienie haploidentyczne może być zdefiniowane jako rodzaj przeszczepienia z użyciem komórek krwiotwórczych pochodzących od dawcy rodzinnego, który jest zgodny jedynie w połowie antygenów HLA. W ostatnich latach opracowany został nowy sposób przeszczepienia haploidentycznego, z zastosowaniem deplecji komórek CD3/CD19, oparty na wykorzystaniu metody immunomagnetycznej z użyciem kulek anty-CD3 i anty-CD19 i separatora CliniMACS. Dzięki tej metodzie, materiał przeszczepowy zawiera nie tylko komórki krwiotwórcze CD34-dodatnie, ale również inne komórki progenitorowe CD34-ujemne, komórki *natural-killer*, komórki dendrytyczne i inne ułatwiające wszczepienie komórek krwiotwórczych i rekonstytucję hematologiczną. W pracy przedstawiono opis przypadku dziecka, u którego wykonano przeszczepienie haploidentyczne z deplecją CD3/CD19, jak również dokonano przeglądu piśmiennictwa odnoszącego się do zastosowanej metody. Chłopiec, 3 lata, leczony z powodu bardzo wczesnej wznowy ostrej białaczki limfoblastycznej, otrzymał kondycjonowanie oparte na: dożylnym busulfanie, fludarabinie, cyklofosfamidzie oraz tymoglobulinie. Pomimo że redukcja liczby komórek CD3 wyniosła 99,9% (tj. 3,64 log), z powodu zbyt dużej ilości limfocytów CD3 w przeszczepie ($2,7 \times 10^5$ CD3+/kg) zastosowano profilaktykę choroby przeszczep przeciwko gospodarzowi (GVHD) z użyciem cyklosporyny A i metotreksatu. Jedynym istotnym powikłaniem po transplantacji była reaktywacja zakażenia CMV, opanowana terapią wyprzedzającą. Badanie szpiku kostnego w dniu +29 wykazało normalną hematopoezę oraz 95-procentowy chimeryzm dawcy. W dniu +300 pacjent pozostaje w remisji. Podsumowując, opisany przypadek poparty przeglądem literatury pokazuje, że przeszczepienie haploidentyczne z deplecją CD3/CD19 jest efektywną i bezpieczną terapią dla dziecka z bardzo wczesną wznową ostrej białaczki limfoblastycznej.

Słowa kluczowe: przeszczepienie komórek krwiotwórczych, dzieci, deplecja CD3/CD19, ostra białaczka limfoblastyczna.

by a high incidence of GVHD, engraftment failure and infectious complications resulting in unacceptable treatment-related morbidity and mortality. Graft rejection and GVHD are primarily mediated by host and donor T cells. Attempts to overcome the HLA barrier have therefore focused on strategies for effective host and graft T-cell depletion. However, even after effective T-cell depletion the success rate of these transplant approaches was dismal. A different strategy for successful HHCT was pioneered by Bachar-Lustig *et al.* [1] in close collaboration with the group of Aversa *et al.* in Perugia [2]. They were able to show first in mice and later in man that the rejection of T-cell depleted bone marrow cells can be overcome by increasing the stem cell dose to a "megadose" (i.e. $\geq 10 \times 10^6$ CD34+ cells/kg) of CD34 positive cells [1, 3].

Based on the promising experiences gained at St. Jude Children's Research Hospital, Memphis, in the paediatric population [4-6], a new regimen using graft CD3/CD19 depletion with anti-CD3- and anti-CD19-coated microbeads on a CliniMACS device was developed. CD3/CD19 depleted grafts contain not only CD34+ stem cells but also CD34 negative progenitors, natural killer, dendritic and graft-facilitating cells, thereby enhancing engraftment and even enabling HHCT after a reduced intensity conditioning (RIC) regimen.

We here present a case description of a child using CD3/CD19 depleted grafts in a haploidentical stem cell transplant setting and a review of available literature.

Case report

The patient, a 3-year old boy, suffering from acute lymphoblastic leukaemia (ALL), immunophenotype common, was treated according to the international ALL-IC-2002 protocol for the intermediate risk group (due to hyperleukocytosis, no CNS involvement). He reached a complete remission (CR1). However, five months after the initial diagnosis, he experienced a very early isolated bone marrow relapse, and he was switched to the protocol for relapse ALL-BFM-2004 for group S4. He reached a second CR, but was qualified for urgent allogeneic haematopoietic stem cell transplantation (allo-HSCT), as this was the only curative therapy for S4 relapsed patients. Since there was no family donor, no unrelated matched or mismatched (i.e. 8/10 or 9/10 HLA match) donor, and no cord blood unit (with at least 4/6 HLA match) was available, he was qualified for haploidentical allo-HSCT (haplo-HSCT). According to recent data, the patient's mother was selected to be a donor [7]. Before the beginning of the conditioning regimen, he was in good clinical condition (Lansky score 100). He received prophylaxis of mucositis with palifermin (rhKGF, recombinant human keratinocyte growth factor) for 3 days.

Myeloablative therapy

The myeloablative therapy consisted of weight-adjusted intravenous busulfan (4×1.1 mg/kg/day over 4 days, total 16 doses, days –11, –10, –9, –8) plus fludarabine (40 mg/m^2/day, at days –7, –6, –5, –4), and cyclophosphamide (60 mg/kg/day, days –3, –2). He additionally received rabbit thymoglobuline (Genzyme) 8 mg/kg/3 days (days –3, –2, –1).

Donor, stem cell mobilization and collection

The mother was selected as a haploidentical donor. HLA typing was done by standard serological analysis of HLA A and B-loci and high-resolution techniques for HLA DRB1 loci. There was a minor blood incompatibility (patient A Rh-negative, mother 0 Rh-positive). The peripheral blood stem cells (PBSC) were mobilized with granulocyte-colony stimulating factor (G-CSF; Neupogen, Amgen, Germany) using 10 µg/kg for 4 days. Collection was done at day 5 using a Cobe Spectra (Cobe, Lakewood, CO, USA). A total of 12 l of blood was processed at a flow of 50-60 ml/min to obtain the final PBMC (peripheral blood mononuclear cells) apheresis product.

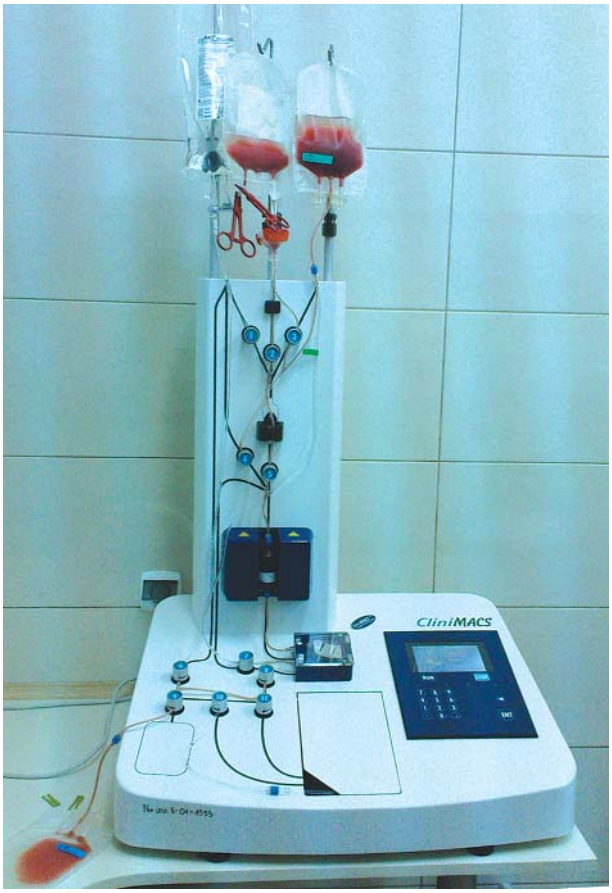

Fig. 1. CD3/CD19 depletion performed by negative selection on CliniMACS device
Ryc. 1. *Deplecja CD3/CD19 w procesie selekcji negatywnej na CliniMACS*

CD3/CD19 depletion

CD3/CD19 depletion was performed by negative selection using the automated CliniMACS device (Miltenyi Biotec, Bergisch-Gladbach, Germany) (Figure 1) according to the manufacturer's protocol. PBMC were processed immediately after the collection. The cells were concentrated to a maximum volume of 150 ml, washed once with CliniMACS PBS buffer (phosphate-buffered saline supplemented with 1 mM EDTA (ethylenediaminetetraacetate) and 0.4% of human albumin, in order to remove platelets) and incubated with anti-CD3 and anti-CD19 antibodies directly conjugated to magnetic microbeads (Miltenyi, Bergisch-Gladbach, Germany). The amount of antibody used was calculated according to the manufacturer's instructions. One vial (7.5 ml) of CD3 and one vial (7.5 ml) of CD19 beads per 4×10^{10} MNC total cells with a maximum of 15×10^9 CD3+ T cells and a maximum of 5×10^9 CD19+ cells were used. The cells were incubated under continuous agitation at room temperature for 30 minutes, washed once with CliniMACS buffer, and resuspended in 150 ml of buffer per 4×10^{10} total cells. The manufacturer's original protocol was eventually modified by addition of 0.15 percent human immunoglobulin G (IgG; Gammagard S/D, N.V. Baxter, Lessines, Belgium) before anti-CD3 microbead incubation, for blocking of non-specific binding [8].

Cell processing

The cells were processed with the fully automated CliniMACS device (Miltenyi) using the software program D3.1 (Depletion 3.1) for CD3/CD19 depletion according to the manufacturer's instructions [8]. Direct B- and T-cell depletion was performed with the depletion tubing set (DTS, 261-01) separation column (labelled cell capacity, 4×10^{10}; maximum processing time, 1.5 hours). The D3.1 program performs a second "sensitive loading stage" after the first round of rapid bulk T-cell depletion to deplete any remaining labelled cells.

Flow cytometry

Analysis of the initial leukapheresis product and CD3/CD19 depleted grafts was performed using flow cytometry. Cells were analyzed for CD3, CD20, CD56 and CD34 expression with fluorochrome labelled antibodies. Antibodies for detection and selection-depletion that bind to different epitopes were chosen and thus the selection-depletion antibody did not interfere with the analysis of the final product. Samples were prepared in duplicate. Exactly 100 µl of thoroughly homogenized cell suspension were stained with 20 µl each of fluorescein-isothiocyanate (FITC) conjugated anti-CD45 monoclonal antibody (mAb) (BD Biosciences, Heidelberg, Germany) and of phycoerythrin (PE) conjugated anti-CD3 mAb (clone SK7, BD Biosciences), which recognizes a different binding site than the T cell depletion mAb. Control samples stained with anti-CD45-FITC (clone J.33) and the appropriate isotypic PE antibodies (BD Biosciences) were used to exclude unspecific staining [9]. To distinguish between dead and vital cells, 20 µl of propidium iodide (Sigma) in concentration of 1 µg/ml was added to each tube. Samples were incubated for 20 minutes in darkness at room temperature. Erythrocytes were lysed with 2 ml of ammonium chloride lysing solution and then 500 µl of PBS were added. The samples were then stored at 4°C in darkness until measurement within one hour. All tubes were then immediately vortexed for 5 s. Either a minimum count of 100 CD3+ events and at least 500 thousand CD45+ events ("live gate" method) were acquired or data acquisition was terminated after 10 minutes. In parallel, samples were prepared for conventional T cell analysis.

Flow cytometric analysis was performed using an FC500 Cytomics four colour-flow cytometer (Beckman-Coulter, Miami, USA) equipped with a 488 nm argon laser and 525, 575, 620, 675 nm band pass-fluorescence filters and the FCX software. All procedures were performed according to the ISHAGE (International Society for Hematotherapy and Graft Engineering) guidelines [10]. Debris, dead cells, aggregates and platelets were excluded by gating on forward and side light scatter and subsequently on CD45+ propidium iodide negative cells.

Stem cell transplantation

Haplo-HSCT was performed from mother PBSC. A total of 14.4×10^8 mononuclear cells/kg of recipient were infused,

including 7.2 × 10^6 CD34+ cells/kg and 2.7 × 10^5 CD3+ lymphocytes/kg. The reduction of CD3 load was 99.9% (i.e. 3.64 log scale).

Graft-versus-host disease prophylaxis. Due to insufficient reduction of CD3 load in the graft (expected < 1 × 10^5 CD3+/kg, current 2.7-fold higher), GVHD prophylaxis was done with cyclosporine A (3 mg/kg/day *i.v.*) and methotrexate (10 mg/m^2 *i.v.*, days +1, +3, +6 and +11). In case of the number of lymphocytes being <1 × 10^5 CD3+/kg in the graft, no GVHD prophylaxis was planned (except ATG, as given during the conditioning regimen) according to the protocol "ALL SCT BFM international: Allogeneic stem cell transplantation in children and adolescents with acute lymphoblastic leukemia" (Vienna, 2007, EudraCT number: 2005-005106-23).

Engraftment and monitoring of infectious complications. A subfebrile state or fever was observed between day –3 up to +12, with general good condition, and negative microbiology tests. Broad spectrum antibiotics were used to treat initial episodes of febrile neutropenia. Due to CMV-DNAemia, patients were given ganciclovir (5 mg/kg/dose *i.v.*). No severe mucositis (grade III or IV) was observed, but TPN was provided up to day +19, due to loss of appetite. Neutrophil (ANC > 500/µl) and platelet (PLT > 20 000/µl) recovery occurred at day +14 and +12, respectively. Bone marrow biopsy at day +29 revealed normal haematopoiesis and donor chimerism 95%. He was discharged from the hospital at day +30 and remains in remission at day +300. The only complication after discharge was CMV reactivation.

Discussion and review of the literature

The basis for the clinical application of haploidentical transplantation was laid by Reisner *et al.* in two major approaches using animal models with lethally and sublethally irradiated mice: escalation of haematopoietic progenitor cell dose and the use of non-alloreactive T cells [11, 12]. Cells within the human CD34+ population are known to have veto activity, which allows the development of a new immune system and the gain of tolerance in spite of HLA disparities [13]. The role of MHC-specific inhibitory receptors that allow NK cells to discriminate between normal cells and potentially harmful cells that have lost or express insufficient amounts of MHC class I molecules are well known today. Donor versus recipient NK-cell alloreactivity can eliminate leukaemia relapse and graft rejection, facilitate engraftment and protect patients against GVHD [13].

The early complications of severe graft-versus-host disease (GVHD) after non-T-depleted (T-repleted) haplo-HSCT, and graft failure and recurrent malignancy (after T-cell depleted SCT) have limited the applications of this approach, in spite of high dose conditioning for haplo-HCT [14, 15]. However, this kind of approach is still complicated by a high TRM due to the intensive conditioning regimens used and the delayed immune reconstitution. Newer strategies employing T- and B-cell depletion of the graft, using very high-dose peripheral blood stem cells, have overcome some of the problems of conventional transplantation [5, 8, 16].

The risk of severe GVHD depends on the degree of HLA disparity, and a rate of up to 90% acute GVHD III-IV was observed in patients receiving unmanipulated marrow grafts from three loci mismatched (i.e. 3/6) related donors. Alloreactive donor T cells have been shown to play a major role in mediating GVHD in this setting. Depletion of these cells can effectively prevent clinical symptoms even in the case of full haplotype mismatched donors. Several strategies have been used to minimize the number of donor T cells in the graft [17]:

- direct depletion of T cells with soybean lectin agglutination *in vitro* or with MoAb *in vitro* and *in vivo*,
- positive selection of CD34+ or CD133+ progenitor cells with immunomagnetic microbeads *in vitro*, which includes indirect depletion of all other cell types,
- direct depletion of T cells with anti-CD3 coated microbeads, in combination with Ab mediated (anti-CD20, rituximab) depletion of B cells *in vivo* or with anti-CD19 coated microbeads.

The number of residual T cells in the grafts is crucial and depletion factors in the range of 4-5 log are usually required, although this is difficult to achieve. There is clinical evidence that even very low cell numbers (3 × 10^4/kg bodyweight) can cause severe GVHD in patients who receive no further post-transplant pharmacological immunosuppression [17].

The recent insight that grafts selectively depleted of T and B cells may contain significantly more graft facilitating cells such as NK cells, monocytes and granulocytes in addition to CD34 positive stem cells gives a rationale for improved engraftment and immune reconstitution. Profound T-cell and B-cell depletion is a fundamental prerequisite for haplo-HCT to avoid severe GVHD and EBV-related post-transplant lymphoproliferative disease (PTLD). Recent studies have revealed the existence of CD34-negative stem cells with repopulating capacity which are likely precursors of CD34+ stem cells [18]. Graft facilitating cells such as CD8 positive T cells, but also NK cells, monocytes and antigen presenting cells (APCs), have been defined [19-22]. No minimal number

Table 1. Available studies on CD3/CD19 depleted haploidentical HSCT
Tabela 1. *Analizy przeszczepień haploidentycznych z zastosowaniem deplecji CD3/CD19*

Source	Centre	Patients	T-cell depletion	Outcome
Handgretinger *et al.* [25]	Memphis	25 children		9/25 alive, at median 18 months
Lang *et al.* [16] Handgretinger *et al.* [25]	Tubingen	11 children (total 29 children)	3.9 log	5/11 alive, at median 8 months
Lang *et al.* [17]	Tubingen	19 adults	4.4 log	35% alive, at median 12 months
Koehl *et al.* [9]	Frankfurt	15 children	0.004%	

of T lymphocytes which enables graft facility in the haploidentical setting has been determined in the literature so far. However, in the study of Aversa *et al.*, as few CD3 cells as 0.8×10^4/kg was sufficient for haematological recovery (mean 2.7×10^4/kg, range $0.8\text{-}7.5 \times 10^4$/kg) [23].

Although haplo-HSCT in acute leukaemia has been performed since 1980 [24], the use of CD3CD19-negative selection is a relatively new approach. There are only 4 reports indexed in the PubMed database, including 70 patients treated with his method (Table 1), mainly based on conditioning consisting of fludarabine, thiotepa, melphalan and anti-CD3 MoAb (muromonab).

Polish experience in haploidentical transplantations with CD3/CD19 depletion was started in Wrocław and Lublin in 4 children with acute leukaemia [26] and 5 children with SCID [27].

We can conclude from our observation and literature review that HLA haploidentical peripheral blood stem cell transplantation with CD3/CD19 depletion may be an effective and safe therapy for children with relapsed leukaemia.

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