

Selected issues regarding cell therapies in light of reports presented at the 33rd Regional Congress of the International Society of Blood Transfusion (ISBT) in Gothenburg, June 17–21, 2023

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Introduction

The International Society of Blood Transfusion (ISBT) is a leading society combining a global community of professionals who share the knowledge on clinical and laboratory transfusion, as well as the safety of blood and blood components for the welfare of blood donors and patients. Every two years ISBT organizes international and regional congresses. On June 17–21, the 33rd Regional Congress was hosted by Göteborg (Sweden). This congress was organized jointly with the Swedish Society for Clinical Immunology and Transfusion Medicine (KITM, Svensk Förening för Klinisk Immunologi och Transfusionsmedicin). The congress agenda included: Nordic Day (June 17), during which reports from five Scandinavian countries were presented, Academy Day (June 18), during which educational lectures covering practical aspects of the most recent topics in transfusion medicine were presented, three days (June 19–21) of sessions presenting the latest reports in the field of transfusion medicine and the related fields. This paper presents some selected issues related to cell therapies presented during the Congress.

Cell therapies

Three lecture sessions and part of the poster session of this year's ISBT Regional Congress were devoted to cell therapies, including hematopoietic stem cell (HSC) transplantation and chimeric

antigen receptor T-cell (CAR-T) therapy. One of the ISBT working parties is dedicated to cell therapies and its purpose is to increase awareness of cell therapies, to support their advancement and to improve the quality of cell therapies for the benefit of patients. The group has 4 projects underway: artificial tears (EDHO, Eye Drops of Human Origin), platelet lysates (Human Platelet Lysate — Current Standards and Future Developments workshop), cell therapies with unproven effectiveness (Unproven Cellular Therapies) and a sub-group dealing with the development of guidelines for stem cell processing based on GMP (Guide to set up a GMP facility for Stem Cell Processing). One of the great advantages of the stationary Congress was the possibility of networking with practitioners dealing with cell therapies on daily basis, who also operate within the aforementioned working group. On Academic Day, the ISBT Young Professionals Council (YPC) hosted a Young Professionals networking breakfast during which groups divided by fields of interest discussed various aspects of their scientific activity with experts and other young professionals. As many as two of the six groups were focused on the topic of cell therapies and shared their experience under the supervision of Denese Marks and Katharina Schallmoser who acted as experts. The first day of the main scientific program was opened by Meet the Experts sessions — Cellular Therapies led by Reinhard Henschler and Mickey Koh. The topics discussed included, among others, the current state and advances in

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cell therapy and new cell therapy products based on various blood components. Many of the papers presented at the Congress, mentioned the COVID-19 pandemic in reference to the difficulties in performing blood collection, transplantation or donor recruitment procedures.

Abstracts on cell therapies submitted to the Congress were divided into 4 groups: 1) stem cell and tissue banking, including umbilical cord blood, 2) collection, processing, storage and issue, 3) clinical applications, 4) tissue compatibility in stem cell transplantation.

Due to the large number of reports both in the lecture and poster sessions, this paper focuses only on the most interesting works devoted to hematopoietic stem cell transplantation, CAR-T, modern cell therapies and hematopoietic cell donors.

Lecture sessions

One of the sessions organized by ISBT in cooperation with the AABB (Association for the Advancement of Blood & Biotherapies) concerned the harmonization of apheresis material for use in cell therapies.

Schwartz (Tampa, USA) drew attention to the differences in the requirements for cell harvesting for the purpose of obtaining lymphocytes for CAR-T therapy. More and more clinical trials are conducted worldwide to test the effectiveness of CAR. There are also newly registered CAR-T drugs for various indications (in the USA there are currently 6 products approved by the FDA). One of the key elements related to CAR-T is the effective collection of lymphocytes as well as the problem of no well-defined apheresis criteria [1]. Schwartz presented the results of a study initiated, among others, by the American Society for Apheresis — the analysis included 621 clinical trials using CAR-T therapy. The aim of the analysis was to collect and evaluate the variability of descriptions referring to collection of material by apheresis. Apheresis as such was mentioned in only 51.9% of study reports. There are also discrepancies in the guidelines regarding the procedure of apheresis — some sponsors/manufacturers require apheresis to be based on a specific duration of the procedure (apheresis), total blood volume (TBV), leukocyte (WBC) count or CD3+ lymphocyte count. The laboratory parameters mentioned in the descriptions and checked in the collected preparation included WBC (100 tests), absolute neutrophil count (220 tests), absolute lymphocyte count (102 tests), CD3+ cells (38 tests), hemoglobin (233 tests)

and platelets (269 tests). Laboratory parameters useful for apheresis cell collection described in the analysed studies vary and are inconsistent with current practices [2]. Each company responsible for a given Advanced Therapy Medicinal Product (ATMP) or CAR-T medicinal product has its own quality control criteria. Only a small percentage of centres determine CD3+ cell yield in the final product and this parameter seems to be crucial for product quality. Schwartz pointed to the need of studying the expression of the CD3 surface antigen as the best indicator of the quality of the material collected for CAR-T.

During Schwartz's lecture, a discussion ensued on logistical problems of centres which collect cells for CAR-T therapy, the differences in cell cryopreservation methods and quality tests as well as the multitude of audits, and also protocols/procedures for each ATMP/CAR-T medicinal product. Moreover, preparations that serve as material for CAR-T are marked with various ISBT128 codes. All these aspects complicate the work of the manufacturing centres and cell banks involved in the procedure and may contribute to higher risk of error. It seems necessary therefore to develop a uniform standard for collecting material for CAR-T production in order to facilitate the work of the collecting and/or processing staff as well as to minimize the risk of error and to limit the scope of documentation related to a given ATMP/medicinal product.

It was interesting to listen to Schafer's (Freiburg, Germany) lecture on the collection of material from allogeneic donors. Currently, lymphocytes/mononuclear cells as the starting material for the production of CAR-T, are obtained mainly from autologous donors — patients suffering from haematological and oncological malignancies. Such procedure of collecting the material from the patient has however numerous drawbacks and is work-intensive and costly. Therefore new methods of producing CAR-T from allogeneic donors are being developed. Precise genome editing may lead to the production of allogeneic immune cell therapies. During Schafer's presentation, the impact of the patient's and donor's health status and individual characteristics on the quality of product were discussed, as well as the practical issues related to donor management (intervals between donations, deferrals or donor identification and eligibility). Additionally, some aspects of material collection techniques for bone marrow-derived cell therapies were discussed as well as the need for cryopreservation and its impact on the quality of

the material [3]. Two studies by Mauer et al. were also presented during this session. They described a lower incidence of GvHD (graft-versus-host disease) after transplantation of cryopreserved allogeneic HSCs from an unrelated donor. The study also demonstrated an insignificant effect of cryopreservation on the quality of HSCs and the final effect of transplantation. The study was performed after the implementation of guidelines on obligatory cryopreservation of allogeneic graft material as consequence of the COVID-19 pandemic [4, 5].

During the other sessions devoted to cell therapies, reports on new therapies were presented. Since the discovery of CAR, which is one of the greatest achievements of the last decade, still ongoing is the search for new methods of CAR therapy in hematological, oncological and other diseases. There is a lot of talk about CAR-NK, which makes use of natural killer (NK) cells instead of T cells. Since NK cell-based immunotherapy requires a relatively large number of cells, which is not so easily done, attempts have been made to develop new methods of obtaining the material. Moazzeni et al. (Tehran, Iran) suggested isolating NK cells from leukoreduction filters (LRF) to recover CAR-NK cells directed against the B-cell maturation antigen (BCMA) used in multiple myeloma patients. The cells retained in the filters are usually disposed of together with the filter. In their study, leukocytes were isolated from the LRF using a special wash and NK cells were purified using the MACS column separation kit. After isolation, the proliferative capacity and cytotoxic effect of the obtained NK cells as well as the presence of surface antigens were assessed. NK cells were then modified by lentiviral transduction with vectors comprising the second-generation anti-BCMA CAR construct. The rate of transduction and the anti-BCMA construct expression on transduced NK cells were assessed using appropriate antibodies. The expression of CD107a, CD16, CD56, IFN- γ and granzyme B was performed by flow cytometry and PCR. The isolation yield was $85 \times 10^6 \pm 5.7$ cells/filter, which is a satisfactory result. The isolated cells were of good quality with highly preserved NK cell proliferation and cytotoxic capacity [6].

Fernandez-Rodriguez (Oviedo, Spain) presented the results of the Phase II clinical trial EudraCT 2008-003015-12, which focused on the treatment of pressure ulcers/sores with the ATMP product containing mononuclear cells (MNC). Bedsores are defined as local damage to the skin and subcutaneous tissue that occurs as a result of intense and

prolonged pressure and/or friction. Because of high infection risk, these wounds are a significant cause of mortality among immobile people. The typical method of treatment is surgery but it is burdened with the risk of failure or recurrence. The clinical trial was designed to evaluate the safety and efficacy of cell therapy in 92 patients with stage III/IV bedsores. Autologous bone marrow was collected from patients for obtaining ATMP and diluted 1:1 in saline with 100 UI/ml heparin and MNCs were isolated using Ficoll. At least 50×10^6 MNCs were thus obtained, which were then resuspended in heparinized saline and subjected to filtration. The material (volume of 10–12 ml) was applied to the sutured wound, which had been cleaned of necrotic tissue. The average number of MNC cells in the product was 104.42×10^6 . Significant differences were observed between the control group (surgical treatment only) and the study group (ATMP) in the percentage of wound dehiscence at 6 months and 1 year after surgery. The comparison was in favour of conventional treatment and the mean hospital stay was in favour of ATMP. Mononuclear cell treatment minimizes the length of hospital stay, which is cost savings, but long-term results still show conventional treatment to be more effective [7].

Khan (Birmingham/Oxford, UK) presented the outcome of his study during which he created a model of a bone marrow organoid that faithfully mimics human tissue. Such models are used to study normal and pathological haematopoiesis in vitro in the bone marrow microenvironment. The model of the marrow organoid was derived from human induced pluripotent stem cells. The resulting cytokine-exposed cell cultures contained inter alia stromal cells, myeloid cells and their progenitors cells. Cells grown in this way included megakaryocytes, erythroid cells, monocytes and granulocyte progenitors. The resulting stroma comprised a branched, three-dimensional network of endothelial cells supported by fibroblasts and mesenchymal-stromal cells. The model not only allowed to test the impact of inflammatory stimuli on the medullary cavity, but also to implement cells from healthy donors and patients with hematologic malignancies (including multiple myeloma, myelofibrosis and acute lymphoblastic leukaemia) in order to create an ex vivo system for the study of haematopoiesis [8].

Poster session

During the congress, the discussions covered many topics related to blood donor recruitment,

eligibility and safety as well as quality of haematopoietic cells. One of the posters presented the results of follow-up of 56 peripheral blood stem cell (PBSC) and bone marrow (BM) donors (23 women and 17 men; median age 36 years (22–52)). Prior to collection, PBSC donors were mobilized with G-CSF at a dose of 12 µg/kg/day; in 86% it was a 5-day mobilization. The most commonly reported symptoms associated with the donation were: systemic and local myalgia, nausea, and pain at the puncture site. Poor exercise capacity and weakness were also observed (more common in BM than PBSC donors). One month after donation, 14% of BM donors and 13% of PBSC donors still complained of moderate pain. 95% of PBSC donors reported no symptoms after 1–5 days of recovery. In the group of bone marrow donors, it was 6–15 days [9].

The Korean Red Cross Blood Service (KRCBS) team presented the results of a 4-year analysis of hematopoietic cell donor recruitment. Since the establishment of Korean stem cell donor registry in 1994, a total of 420,217 potential stem cell donors have been registered 37.8% of which were donors registered with KRCBS. In 2022, 50.9% of qualified donors came from the KRCBS registry. KRCBS focuses on the so-called individual recruitment conducted by qualified nursing staff in blood donation centres. This program is targeted at repeat blood donors — 99.9% of registered potential stem cell donors were also blood donors. The study includes an analysis of the effectiveness of the individual recruitment program as compared to group recruitment and a comparison of KRCBS results with that of other organizations recruiting donors in the years 2019–2022. During the pandemic, organizations relying on group recruitment demonstrated lower effectiveness, which is why KRCBS played a significant role in recruiting new potential stem cell donors when group recruitment was limited due to the COVID-19 pandemic [10].

Useini et al. (Skopje, North Macedonia) presented their experience in collecting PBSC by apheresis from both patients and healthy donors. Patients and donors were mobilized with G-CSF (Granulocyte-Colony Stimulating Factor). The minimum target of apheresis was to collect 2×10^6 /kg CD34+ cells or 2×10^8 /kg MNCs. PBSC separation was carried out using Baxter CS3000, COBE Spectra and Spectra Optia cell separators with ACD-A anticoagulant. The TBV processed during apheresis was within the range of 2.0–2.5. Between 2000 and 2022, their centre performed 977 PBSC (Peripheral Blood Stem Cell) apheresis

procedures, of which 81% were for autologous patients and 19% for healthy donors, including 5 unrelated donors. A single apheresis procedure lasted from 180 to 270 minutes, and the volume of preparations ranged from 50 to 400 ml. The most common indications for autologous transplantation were multiple myeloma (51.1%), acute myeloid leukaemia (18.5%), non-Hodgkin's lymphoma (13.6%), and Hodgkin's lymphoma (13.4%) while the indications for allogeneic transplantation were mainly: acute myeloid leukaemia (55.8%), acute lymphoblastic leukaemia (14.2%), chronic myelogenous leukaemia (7.5%), severe aplastic anaemia (5.8%), myeloproliferative diseases (5%) [11].

The routinely applied method of maintaining cell viability for an extended period is the aforementioned method of stem cell cryopreservation. For this purpose, a cryoprotective mixture is added to the cells to protect them from extracellular and intracellular crystal formation. This mixture typically contains a 5–10% concentration of dimethyl sulfoxide (DMSO) as cryoprotectant, along with plasma, albumin, or hydroxyethyl starch (HES), depending on the protocol used in that particular centre. Due to either temporary market shortages of certain reagents or following changes in guidelines, centres are focused on developing new methods of cryopreservation. Pursuant to suspension of HES production by EU countries, Jacobsen et al. (Trondheim, Norway) decided to validate the cryopreservation process at freezing conditions different from the previously used. The aim of the validation procedure was to establish freezing curves for the cryopreservation method with 5% DMSO with no HES in a –80°C freezer, and to compare the results for cell viability in frozen samples with HES. Prior to thawing and analysis the material and samples were stored in liquid nitrogen vapor for a week. After thawing, the cell viability in the stem cell preparations and the collected samples was examined. The cell viability averaged 99.9% and 98.5% in the products and samples frozen with 5% DMSO and HES respectively and 99.7% and 98.8% when they were frozen using 5% DMSO. The obtained results confirm that both freezing methods yield similar and high cell viability [12].

The toxic effects of DMSO and the numerous side effects observed in patients after transplantation of cryopreserved stem cells encourage cell banks to improve their preparation procedures. This includes testing various modifications of the qualitative and quantitative composition of cryoprotective mixtures, as well as the solution used for DMSO rinsing after thawing. In their

study, the Al-Mozain team (Riyadh, Saudi Arabia) attempted to assess the impact of reduced DMSO concentration (from 10% to 5%) in the cryoprotective mixture on cell viability. The study was performed on 10 random preparations of PBSC (Peripheral Blood Stem Cells) and bone marrow. Each donation was subjected to cryopreservation in two variants: using 5% and 10% DMSO concentrations. The qualitative parameters assessed in the products before cryopreservation and at 2, 4, and 8 weeks after thawing were: WBC count, total number of CD34+ cells, and cell viability. The results at 2, 4, and 8 weeks after cryopreservation clearly indicated that in preparations with lower DMSO concentrations, cell viability was higher. The validation results encouraged the centre to consider the 5% DMSO method as the new standard for cryopreservation of paediatric stem cell preparations; the potential adverse effects of large amounts of dimethyl sulfoxide can be particularly serious in paediatric patients [13].

The administration of thawed stem cell preparations with DMSO as a cryoprotectant is recommended within 10–15 minutes of thawing because at room temperature, dimethyl sulfoxide may adversely affect the cells. For this reason, many centres remove DMSO before administering stem cells to the patient. Some of the methods of DMSO removal include rinsing with human serum albumin (HSA) and dextran or albumin and HES. In their study, Larrea et al. (Valencia, Spain), described only HSA used for rinsing stem cells because of the aforementioned market shortages of HES and dextran. The study included 26 stem cell preparates destined for disposal. All of them met the criteria of final cell concentration of $< 3 \times 10^8$ cells/ml as well as cryopreservation with 10% DMSO within 24 hours of collection under controlled freezing conditions. The containers were thawed in a water bath at 37°C. After thawing, a rinsing solution containing HES-HSA (control group) or HSA solution (experimental group) was added to the stem cells. In each final product, the total nucleated cell (TNC) count, viability, and clonogenicity were assessed immediately after rinsing and 60 minutes later. No significant differences in the quality parameters of the tested preparations were observed between the groups, thus the HSA solution was deemed equivalent to the HSA-HES mixture [14].

Summary

Numerous reports presented during the 33rd ISBT Regional Congress demonstrate the dynamic

changes in the field of cell therapies and show how much still requires investigation. Every year brings a growing number of reports on the improvement or development of new life-saving cell therapies. Centres involved in the collection and processing of material must be prepared for dynamic changes related to evolving standards, material and reagent shortages, as well as necessity to ensure adequate quality and safety measures. Standardization is also required in the area of guidelines related to lymphocyte collection for CAR-T therapy, which is still a significant challenge for laboratory, nursing, and medical staff. A critical aspect of allogeneic material collection is the donor himself who needs to be well-guided throughout the entire process, from recruitment to post-donation.

Conflict of interest: none declared

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