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**Letters to the Editor**

# Quantum cell expansion system: Safe and rapid expansion

ILKNUR KOZANOGLU[1], ERKAN MAYTALMAN[1], CIGDEM GEREKLIOGLU[2], MAHMUT YERAL[2], NURHILAL BUYUKKURT[2], PELIN AYTAN[2], CAN BOGA[2] & HAKAN OZDOGU[2]

[1]*Cell Processing, Baskent University Adana Adult Bone Marrow Transplantation Center, Yuregir, Adana, Turkey, and* [2]*Clinical Unit, Baskent University Adana Adult Bone Marrow Transplantation Center, Yuregir, Adana, Turkey*

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*To the Editor:*

In recent years, mesenchymal stem cells (MSCs) have been obtained from various tissues. MSCs have been using safely in many diseases, particularly in graft-versus-host disease (GVHD) after allogeneic stem cell transplantation. The most important limitations in the clinical use of MSCs are Good Manufacturing Practice (GMP) requirements for culturing and the long time periods required for culturing methods. In addition, culturing for too many passages and the presence of hundreds of manipulations are important factors that may affect the standardization of the cells. Conventional flask-based culture is mostly used for MSC production. However, flask-based culture expansion is time- and labor-intensive with many open processing steps. A potential solution is the use of automated, closed and GMP-compliant devices in the form of bioreactors.

Allogeneic stem cell transplantation (SCT) from a human leukocyte antigen (HLA)-identical sibling donor after a non–myeloablative-conditioning regimen is a powerful treatment choice in adult aplastic anemia [1]. However, the benefits of this treatment are diminished by complications such as GVHD, which can be life threatening. In cases in which GVHD is refractory to steroid treatment, the lack of effective therapies has bolstered clinical evaluation of MSC therapy for GVHD [2]. The production of MSCs, however, for clinical use can be burdensome, requiring extensive resources, space, labor and GMP compliance. In recent years, the use of bioreactors has become increasingly popular as an alternative to more traditional open systems [3–6]. We discuss here a patient who was treated with MSC cultured in a closed system bioreactor after allogeneic stem cell transplantation.

A 24-year-old patient with aplastic anemia underwent a successful allogeneic stem cell transplantation from an HLA sibling donor with a nonmyeloablative regimen, consisting of fludarabine, cyclophosphamide and antithymocyte globulin with cyclosporine and mycophenolate mofetil for GVHD prophylaxis. Two months after the transplantation, the patient had developed grade II skin GVHD [7], which responded to treatment with 2 mg/kg prednisolone and 3 mg/kg antithymocyte globulin. Three months post-transplant, grade III acute skin and liver GVHD recurred but failed to respond to treatment with cyclosporine and steroid. Given the rapidly deteriorating clinical condition, the competent authority was notified, and permission to perform an MSC transfusion was requested. The patient was not treated on a special study but within the scope of a hospital exemption. A written informed consent was then obtained from both the patient and the original donor.

Because of the severity of the clinical situation and the urgent timeline for MSC production, the transplant team decided to use MSCs produced in a bioreactor. The procedure is as follows.

Correspondence: **Ilknur Kozanoglu**, MD, Baskent University Adana Adult Bone Marrow Transplantation Center, Director of Cell Processing Unit, Dadaloglu Mah. Serinevler 01250, Yuregir, Adana, Turkey. E-mail: ipamuk5@gmail.com

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Twenty-five milliliters of bone marrow were aspirated from the original donor. Four liters of media and 4 L of phosphate-buffered saline were loaded into the Quantum (Terumo BCT) machine using the closed-cell expansion set. The cells were continually fed in a manner dictated by periodic lactate measurements and corresponding feed adjustments, as recommended by the manufacturer. After the 14-day primary culture, cells were harvested using trypsin and subsequently cultured further in a second bioreactor. The samples taken from the final product were analyzed for viability, cell count, microbial growth, endotoxin and telomerase enzyme activity. Additional cell quality testing included flow cytometry, lymphocyte proliferation inhibition and differentiation into adipocytes and osteocytes.

The two-passage, 23-day expansion of the 25-mL sample of bone marrow yielded a total of 616 million cells. The cell product was washed using the Sepax automated processing system (Biosafe), after which the cell viability was observed at 99%. No microbial growth was observed, and endotoxin analysis was negative. The cells were negative for CD45 and CD34 and positive for CD73, CD105 and CD90. MSCs cultured with peripheral lymphocytes were shown to inhibit lymphocyte proliferation, and the cells were shown to differentiate into adipocytes and osteocytes. Telomerase enzyme activity was determined to be below 1.5% RTA (relative telomerase activation).

The MSCs obtained using the Quantum Cell expansion system was infused to the patient at two instances with doses of $1.5 \times 10^6$ cells/kg. We did not observe any complications during the infusion and within 24 h after the infusion. We followed the patient at the first and the third month of infusion. No skin lesions were observed, and liver enzymes had decreased. Thus, the GVHD score was decreased from III to I. The patient is currently 100% donor-type chimeric, and he remains in hematologic remission.

Some studies have reported that immunomodulatory capacity of MSCs produced in different laboratories may not be consistent despite GMP compliance [3]. Culturing for too many passages and the presence of hundreds of manipulations are important factors that may affect the standardization of the cells. Bioreactors can be advantageous because of the standardization provided by automation and the potential to maintain a closed system, thus limiting the impact of complex manual manipulations and the potential for contamination. In some instances, such processes have demonstrated that more cells may be obtained in less time while maintaining a high product quality [4–6].

According to international standards, MSCs are advanced cell therapy products, and these cells should be produced in accordance with GMP conditions. Our bone marrow transplant center is Joint Accreditation Committee ISCT and EBMT–accredited, and we perform approximately 100 transplants per year; however, a GMP laboratory is not currently available at our center.

The Quantum Cell Expansion System is a commercial GMP-compliant device. The system provides automated cell culture in a closed, hollow fiber bioreactor. Pre-defined, customizable settings dictate how cells are seeded, continuously fed and eventually harvested [8]. Production with closed and automated bioreactors can also help reduce cost and time requirements, for example, by manufacturing in a lower-grade clean room, plus optimizing the process and personnel (workload) needs of cell expansion) [4–6].

We may encounter GVHD cases because of the high number of transplant activities at our center. However, given that MSCs may be used to treat GVHD refractory to steroid and other immune-suppressive treatments, we suggest that using the bioreactors can help clinicians overcome this challenging condition at institutions where a GMP laboratory is not available. We conclude that the widespread adoption of bioreactors will lead to significant changes in the field.

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