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Tumor Lysate-Stimulated Dendritic Cell Production: A Cross-Sectional Study

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ABSTRACT

Objective: Despite standard treatments (surgery, chemotherapy, and radiotherapy), resistance or recurrence may still develop in malignant diseases. Therefore, immunotherapeutic approaches, particularly dendritic cell–based tumor vaccines, are gaining importance as emerging treatment strategies. This study investigated the feasibility of using blood storage bags as culture systems for dendritic cell production in tumor vaccine preparation as an alternative to expensive Good Manufacturing Practice laboratory conditions. Furthermore, dendritic cell stimulation was evaluated using allogeneic tumor lysate.

Materials and Methods: CD34+ stem cells and mononuclear cells collected from donors via apheresis were combined with allogeneic tumor lysate in blood storage bags. Mature dendritic cell differentiation was induced by adding cytokines and prototypical immunoregulatory cytokines that stimulate bone marrow cell proliferation and support mature leukocyte formation.

Results: Flow cytometric analyses revealed that the targeted levels of mature dendritic cells were not achieved in cultures using blood storage bags. However, subsequent experiments demonstrated that mature dendritic cells could be generated from allogeneic tumor lysate under appropriate culture conditions.

Conclusion: These results suggest that economical and practical culture systems may be developed; however, standardization of culture conditions remains critical.

Keywords: Blood storage bag, culture medium, dendritic cell, immunotherapy, tumor vaccine.

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INTRODUCTION

Surgery, chemotherapy, and radiotherapy have traditionally been the primary approaches for treating malignant diseases. Although these methods provide positive responses in many patients, treatment resistance, relapse after remission, and inadequate responses, particularly in advanced tumors, remain significant clinical challenges. Resistance to chemotherapy and radiotherapy is associated with multifaceted mechanisms, including genetic and epigenetic adaptations of

tumor cells, immunosuppressive characteristics of the tumor microenvironment, and reprogramming of cellular signaling pathways. These factors limit the efficacy of current standard treatment protocols when used alone and necessitate the development of new strategies involving more effective, targeted, immunomodulatory, or combination therapies.[1–3] Current knowledge indicates that the dynamic interaction between the tumor microenvironment and the immune system plays a decisive role in cancer development and progression. Consequently, cell-based immunotherapies have emerged as an important treatment option.[2,4] The primary goal of this treatment approach is to activate immune system cells involved in generating tumor-specific responses, enhance their functions, and increase their capacity to recognize and eliminate tumor cells.[5] Thus, this approach aims to selectively identify and eliminate tumor cells by redirecting the organism's own immune response. Cell-based immunotherapies are becoming increasingly important in cancer treatment because they offer a more flexible and targeted approach to addressing tumor heterogeneity and treatment resistance compared with conventional therapies.[5,6]

Major histocompatibility complex (MHC) molecules play a critical role in initiating this immune response. MHC class molecules process antigenic structures and present them to T cells, thereby enabling cellular immune responses.[7] However, T-cell activation requires not only antigen recognition but also the appropriate presentation of that antigen by professional antigen-presenting cells (APCs).[8] Dendritic cells (DCs) are professional antigen-presenting cells with the highest capacity for antigen processing and presentation and are considered an effective cellular vaccine platform in cancer immunotherapy.[9–11]

For dendritic cell vaccines to be implemented in clinical practice, the manufacturing process must comply with Good Manufacturing Practice (GMP) standards. This includes the preparation of tumor-derived antigens (e.g., tumor lysate) under sterile conditions and the in vitro differentiation, maturation, and antigen loading of dendritic cells from peripheral blood-derived mononuclear cells, all under controlled cell culture protocols.[10,11] The resulting dendritic cells must meet clinical criteria for purity, viability, and functional activity before patient administration.[9] However, this production process requires costly GMP-compliant infrastructure, sterile cell culture equipment such as laminar flow cabinets and CO_2 incubators, and automated cell processing systems to standardize cell isolation and maturation steps. Therefore, dendritic cell vaccine production cannot be routinely implemented in many centers due to factors such as cost, the need for specialized personnel, and limited laboratory infrastructure.[12,13] This study aims to demonstrate that dendritic cell production can be achieved cost-effectively, even without access to specialized laboratory facilities. This approach may increase the accessibility of immune-based tumor vaccines and expand opportunities for future clinical applications and research. Such cost-effective experimental platforms may also facilitate preliminary research in resource-limited settings before transitioning to fully GMP-compliant manufacturing systems.

KEY MESSAGES

- Standard dendritic cell culture conditions are essential for achieving clinically relevant dendritic cell maturation, even when simplified and cost-effective culture systems are used.
- The successful generation of mature dendritic cells using allogeneic tumor lysates supports the translational potential of this approach for dendritic cell–based cancer immunotherapy.
- Optimization of low-cost culture strategies may facilitate broader clinical access to dendritic cell vaccines, particularly in centers with limited GMP infrastructure.

MATERIALS AND METHODS

Study Place and Design

This observational study included adult patients (aged ≥18 years) with malignant hematological disorders who were followed in the Division of Hematology, Department of Internal Medicine, Erciyes University Faculty of Medicine, between [08/2014] and [08/2015]. Age- and sex-matched healthy donors referred to the Apheresis Unit of Erciyes University were also included as controls.

Ethical Approval

This study was approved by the Erciyes University Clinical Research Ethics Committee (Approval Number: 2014/92; Date: 07.02.2014). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patients and Data Collection

Of the five patients included in the study, three were diagnosed with acute myeloid leukemia (AML), one with B-cell acute lymphoblastic leukemia (B-ALL), and one with mantle cell lymphoma (MCL). The patients' ages ranged from 18 to 64 years; four were female and one was male. The donor group consisted of five healthy volunteers, including two females and three males, with ages ranging from 18 to 62 years. Four donors were allogeneic donors, and one was an autologous stem cell transplant donor (Table 1).

Table 1. Patient and donor characteristics

Variable	Patient group (n=5)	Donor group (n=5)
Age (min–max)	18–64	18–62
Sex (F/M)	4/1	2/3
Clinical diagnosis	AML (n=3), B-ALL (n=1), MCL (n=1)	—
Cell collection method	—	Apheresis (peripheral stem cell collection)
$CD34^+$ cell dose ($\times 10^6$/kg)	—	1.0–1.7
Cell viability rate (%)	—	≥99.5

F: Female; M: Male; AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; MCL: Mantle cell lymphoma.

Inclusion Criteria

Participants were eligible for inclusion if they were aged ≥18 years; had a morphologically and immunophenotypically confirmed diagnosis of hematologic malignancy (B-ALL, AML, or MCL) in the relapsed or refractory stage for tumor lysate preparation; or were healthy volunteer donors (related or unrelated) eligible for granulocyte colony-stimulating factor (G-CSF) mobilization and apheresis. Donors were required to have complete apheresis records, adequate post-collection cell viability (≥90%), and sufficient $CD34^+$ and mononuclear cell (MNC) counts. For all samples, comprehensive laboratory data, including flow cytometric evaluation of dendritic cell maturation markers (CD83, CD86, HLA-DR, and CD14), were required.

Exclusion Criteria

Patients and donors were excluded if they had evidence of active viral (HBsAg, anti-HCV, anti-HIV), bacterial, or fungal infection; documented autoimmune or chronic inflammatory disease; or pregnancy or lactation. Donors were additionally excluded if they had received immunosuppressive therapy or cytotoxic agents within four weeks prior to mobilization. Samples were excluded from the final analysis if microbial contamination occurred during incubation, post-apheresis cell viability was <90%, or flow cytometric data for dendritic cell maturation markers were incomplete. Only individuals and samples meeting all eligibility criteria were included in the final analysis.

Clinical, Surgical, and Laboratory Investigations

All clinical and laboratory data were collected prospectively using standardized data collection forms. Demographic characteristics, disease subtype, relapse status, donor type, and apheresis-related parameters were systematically recorded. Laboratory variables included $CD34^+$ cell counts, mononuclear cell yields, cell viability, and flow cytometric markers used to assess dendritic cell maturation. All data were anonymized and processed in accordance with institutional and ethical standards.

Peripheral blood samples were collected from patients to prepare tumor cell lysates, which were subsequently used as antigen sources for dendritic cell maturation. G-CSF (1 MIU/kg/day) was administered for 5 days to mobilize CD34(+) stem cells and mononuclear cells into the peripheral circulation of donors. On the sixth day, CD34(+) stem cells and mononuclear cells were collected via apheresis at a target dose of 1×10^6/kg.

The resulting CD34(+) cell and mononuclear cell suspension was combined with the patient-derived tumor lysate in a blood storage bag under sterile conditions. Granulocyte-macrophage colony-stimulating factor (GM-CSF) (800 U/mL) and interleukin 4 (IL-4) (1000 U/mL) were added to the culture medium to initiate dendritic cell differentiation. The cells were incubated, and samples were collected at 48–72 and 96–120 hours of culture to assess CD83, CD86, CD14, and HLA-DR expression by flow cytometry.

A total of five separate treatments were performed, with each patient's lysate paired with a single donor cell sample. Throughout the culture process, culture conditions (incubation temperature, CO_2 supplementation, and culture medium type) were progressively optimized to enhance cell maturation.

Obtaining Tumor Cell Lysate from Patients

Two 2-mL peripheral blood samples were collected intravenously from patients enrolled in the study during the relapse period using EDTA tubes. The first sample was analyzed by flow cytometry for disease-specific immunophenotypic assessment. After immunophenotyping, the second sample was stored at +4°C for a maximum of 24 hours for use as a tumor cell lysate.

Mechanical or chemical lysis procedures, which are necessary to obtain cells from tumor tissue in autologous tumor vaccine applications, were not applied in this study because malignant cells circulating in peripheral blood are already suitable for lysis. Therefore, no additional processing or culture manipulation was required for blood samples used as tumor cell lysates; the samples were prepared only for direct use in dendritic cell stimulation after short-term controlled cold storage.[14]

Peripheral blood samples containing circulating malignant cells were used directly as tumor antigen sources without additional mechanical or chemical lysis procedures.

CD34(+) Stem Cell and Mononuclear Cell Collection from a Healthy Donor

Healthy donors participating in the study received subcutaneous G-CSF (1 MIU/kg/day) administration for 5 days according to the standard mobilization protocols of the Apheresis Unit at Erciyes University. On the fifth day, complete blood count and peripheral blood CD34(+) cell levels were assessed. Apheresis was initiated when the peripheral blood CD34(+) cell count reached ≥20 cells/μL. Apheresis was performed using the Fresenius apheresis system for allogeneic donors and the Amicus apheresis system for autologous donors. CD34(+) stem cells and mononuclear cells were collected from each donor at a target dose of 1×10^6 CD34(+) cells/kg. The presence and viability of CD34(+) cells in the collected samples were confirmed by flow cytometry according to the Erciyes University Transplant Center CD34(+) Cell Analysis Standard Operating Procedure (SOP No: 196).[15]

Incubation Process Using Blood Storage Bags

To provide a low-cost, closed-system incubation environment for dendritic cell culture, 450 mL polyvinyl chloride (PVC) blood storage bags containing CPDA-1, CPD, or SAG-M anticoagulants were used in this study. All procedures were performed under sterile conditions in a laminar flow cabinet. A suspension containing 1×10^6/kg CD34(+) stem cells and mononuclear cells obtained from donors via apheresis was transferred into the bag according to the measured cell volume. In this study, dendritic cells were generated from donor-derived peripheral blood mononuclear cells containing mobilized CD34(+) hematopoietic progenitor cells collected by apheresis following G-CSF stimulation. Subsequently, 2 mL of tumor cell lysate stored at +4°C was added to the bag.

Before incubation, a 0-hour sample was collected, and CD34(+), CD14, HLA-DR, CD83, and CD86 expression levels were assessed by flow cytometry to establish a baseline profile. GM-CSF (800 U/mL) and IL-4 (1000 U/mL) were then added to induce the differentiation of PBMCs into immature dendritic cells. The bags were incubated under the conditions determined during the optimization phase of the study. During this phase, incubation was ultimately performed at 37°C in a humidified incubator with 5% CO_2, which was identified as the optimal condition for dendritic cell differentiation. According to the literature, dendritic cell maturation progresses from an immature to a mature phenotype at 48–72 hours and 96–120 hours, respectively.[16] Therefore, serial samples were collected from the bags at 48 or 72 hours and 96 or 120 hours, and the maturation process was monitored by flow cytometry through increased CD83 and CD86 expression and decreased CD14 expression.

Flow Cytometric Analysis of Tumor Cell-Stimulated Mononuclear Cells

At the specified incubation time points, 2 mL of samples were collected from the blood storage bag and transferred into EDTA tubes. A 100 μL aliquot of each sample was transferred to a TruCount® tube, and 20 μL of fluorescently labeled antibodies against the surface markers CD14, CD34, CD45, HLA-DR, CD83, CD86, and CD209 were added. After vortex mixing, 500 μL of OptiLyse lysing solution was added to the tubes, and the samples were incubated in the dark for 10 minutes. The cell suspensions were then washed with 2 mL of isotonic solution by centrifugation at 1500 rpm for 5 minutes, and this procedure was repeated three times. The resulting cell pellets were analyzed by flow cytometry. Flow cytometric analyses were performed using a multicolor flow cytometer (Navios™, Beckman Coulter, Brea, CA, USA), and at least 10,000 events were acquired and analyzed for each sample.

The dendritic cell differentiation process was evaluated in three stages:

Hour 0 (baseline): The mononuclear cell phenotype was confirmed ($CD14^+/CD45^+/HLA\text{-}DR^+$), and maturation markers were negative as expected ($CD80^-$, $CD83^-$, $CD86^-$).

Hours 48–72 (immature DC phase): Immature dendritic cell development was assessed through $CD45^+/HLA\text{-}DR^+/CD86^+$ expression, along with a decrease in CD14 expression.

Hours 96–120 (mature DC phase): Maturation was confirmed by an increase in costimulatory markers $CD83^+$ or $CD80^+$ on $HLA\text{-}DR^+$ cells.

A dendritic cell population of ≥60% in flow cytometric analysis was used as the threshold for significant maturation.[17]

Optimization Studies

In this study, sequential optimization procedures were performed to determine the optimal culture microenvironment for DCs obtained from peripheral blood mononuclear cells (PBMCs) stimulated with tumor cell lysate. In each application, parameters associated with negative outcomes were systematically modified:

• Application 1: Lysate from a B-ALL patient + unrelated donor MNCs, with bag incubation at +4°C. Flow cytometric analysis at 48 and 96 hours showed that no mature DCs were detected.

• Application 2: Considering the variability in lysate immunogenicity among different disease groups reported in the literature, AML lysate was used instead. Because DC

Table 2. Flow cytometry findings by application

Application	Tumor lysate source	Incubation condition	CD83 (%)	CD86 (%)	CD14 (%)	Result
1	B-ALL	+4°C	0 → 0	35.6 → 27.6	Unchanged	No conversion
2	AML	+4°C (120 h)	0.01 → 0.09	37.2 → 34.3	↑31.6 → 35.4	No conversion
3A	AML	37°C bain-marie	No increase	No increase	—	No conversion
3B	AML	37°C + 5% CO_2	48 h: —; 120 h: ↑	55.8 → 81.6	↓	Conversion
4A	MCL	37°C bain-marie	0 → 0	0 → 0.06	—	No conversion
4B	MCL	37°C + 5% CO_2	34.4 → 74.8	61.4 → 76.9	↓	Conversion
5	AML (related donor)	37°C + 5% CO_2	4.4 → 15.3	32.2 → 25.6	Unchanged	Poor conversion

AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; MCL: Mantle cell lymphoma.

maturation may require 4–5 days and, in some cases, 6–7 days,[16] the incubation period was extended to 120 hours. Despite this modification, no maturation was observed.

• Application 3: The incubation microenvironment was optimized while maintaining incubation at +4°C.

3A: Incubation at 37°C in a bain-marie using the bag system without CO_2 supplementation.

3B: Incubation at 37°C with 5% CO_2 using standard culture dish conditions.

DC maturation was achieved in condition 3B, with a significant increase in CD83/CD86 expression, whereas condition 3A was insufficient.

• Application 4: The disease group was repeated using MCL lysate, while maintaining the 3A/3B setup. The strongest maturation response (CD83↑, CD86↑, CD14↓) was achieved under 37°C/5% CO_2 conditions; the bain-marie condition was again inadequate.

• Application 5: Unlike the previous applications, a related donor was used. Partial and low-grade maturation was observed under 37°C/5% CO_2 conditions.

In summary, +4°C incubation and CO_2-free bain-marie conditions did not support DC maturation. Incubation at 37°C with 5% CO_2 and transfer to a culture vessel were identified as critical determinants. Furthermore, the lysate source (AML vs. MCL) and donor relationship influenced the results.

Dendritic Cell Culture Protocol (Final and Applied Workflow)

1. Combination and Pre-Stimulation: Tumor cell lysate obtained from the patient was combined with MNCs collected from the donor in a blood storage bag under sterile conditions. DC differentiation was initiated by adding GM-CSF (800 U/mL) and IL-4 (1000 U/mL).
2. Transfer to Culture Medium: Approximately 20 mL of cell suspension was collected from the bag and transferred to Iscove's Modified Dulbecco's Medium (IMDM).
3. Pre-incubation: The suspension was incubated at 37°C with 5% CO_2 for 2–3 hours (adhesion phase).
4. Washing and Continued Incubation: Non-adherent cells were removed twice using PBS, and incubation of the remaining adherent cells was continued for 5 days under 37°C/5% CO_2 conditions.
5. Time Points and Analysis: Samples were collected at 48, 72, 96, and 120 hours, and the immature-to-mature DC transition was monitored by flow cytometry using CD14, HLA-DR, CD83, and CD86 markers.

Statistical Analysis

Due to the methodological nature of the study and the limited sample size, statistical hypothesis testing was not performed. Flow cytometric data were evaluated descriptively, and changes in expression levels over time were presented graphically.

RESULTS

The clinical diagnoses of the five patients included in the study were confirmed by flow cytometric analysis. AML was detected in three patients, B-ALL in one patient, and MCL in one patient. The viability rate of CD34(+) cells obtained by apheresis from the peripheral blood of five healthy donors was >99.5% in all samples (Table 1).

Dendritic cell maturation varied depending on the tumor cell lysate source, incubation temperature, and culture conditions. In the first two applications, which were incubated in blood bags at +4°C, no increase in CD83 or CD86 expression was observed, and mononuclear cells were not found to differentiate into the dendritic cell phenotype (Table 2).

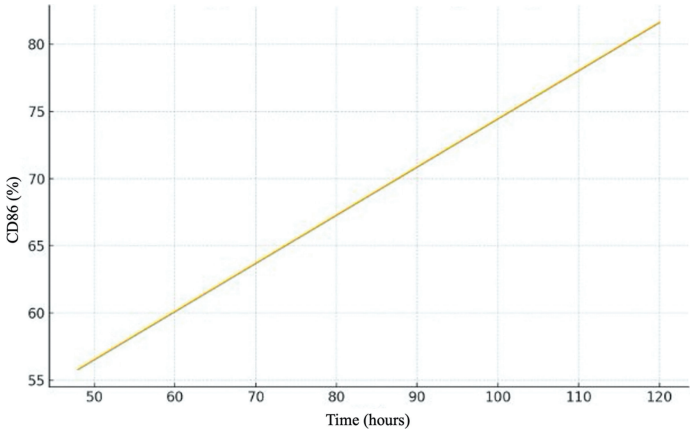

Figure 1. Application-3B: CD86 expression change.

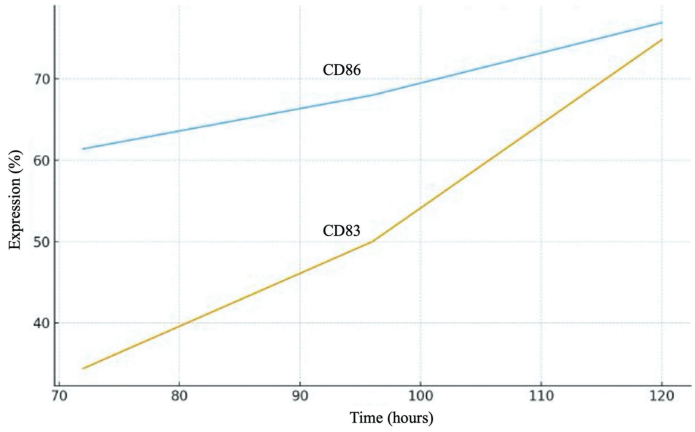

Figure 2. Application-4B: CD83 and CD86 expression change.

In the third application, some of the lysate-exposed cells were cultured in a 37°C bain-marie, whereas the remaining cells were cultured under 37°C and 5% CO_2 conditions. While no maturation was observed in the bain-marie arm, CD86 expression in the sample cultured in the incubator increased from 55.8% at 48 hours to 81.6% at 120 hours, indicating differentiation into dendritic cells (Table 2, Fig. 1).

In the fourth application, the lysate source was changed to mantle cell lymphoma. While no maturation occurred in the bain-marie environment, CD83 expression increased from 34.4% to 74.8% and CD86 expression increased from 61.4% to 76.9% between 72 and 120 hours under 37°C and 5% CO_2 conditions. A significant decrease in CD14 levels was also observed. This application demonstrated the most pronounced mature dendritic cell phenotype in the study (Table 2, Fig. 2).

In the fifth application, when cells from a related donor were used, a limited increase in dendritic cell markers was observed (CD83: 4.42% → 15.36%). The decrease in CD86 levels and the lack of a significant change in CD14 levels indicated that maturation was weaker and slower (Table 2).

Overall, the critical determinant of dendritic cell maturation was concluded to be culture conditions of 37°C and 5% CO_2. Additionally, the tumor type used for lysate preparation and the relative immune compatibility between the donor and patient may influence the magnitude of the response.

DISCUSSION

This study evaluated the use of a blood storage bag as a low-cost, closed platform for the production of DCs from tumor cell lysate-stimulated PBMCs. Different incubation microenvironments (temperature/CO_2), tumor lysate sources (B-ALL, AML, and MCL), and donor relationships (unrelated vs. related) were compared to assess their effects on DC maturation. The findings demonstrated that +4°C or 37°C bain-marie conditions without CO_2 supplementation did not support maturation, whereas the mature DC phenotype, defined by increased CD83/CD86 expression and decreased CD14 expression, was reliably achieved under culture conditions of 37°C and 5% CO_2. This pattern is consistent with classical literature emphasizing the strong dependence of DC biology on microenvironmental parameters, including pH, gas exchange, temperature, and cell-surface interactions.[10,18,19]

The Incubation Microenvironment Determines Maturation

DC maturation is a metabolically demanding process involving antigen uptake and processing, as well as increased expression of costimulatory molecules (particularly CD83 and CD86) and MHC-II (HLA-DR).[18,19] CO_2 supplementation and a temperature of 37°C are essential for the stable function of the bicarbonate buffer system; these conditions maintain cytokine signaling activity (GM-CSF/IL-4) and NF-κB-mediated maturation pathways.[10,18] In our study, the absence of maturation observed at +4°C and the failure of a CO_2-free bain-marie environment suggest that a microenvironment incompatible with these physicochemical requirements suppresses DC differentiation. Conversely, the reproducible confirmation of maturation through increased CD83/CD86 expression and decreased CD14 expression under 37°C and 5% CO_2 conditions reinforces the fundamental principles of standard monocyte-derived DC protocols.[12,20]

Blood Storage Bags are Practical in the Early Stages but Limited for Maturation

Although closed-bag systems offer advantages such as sterility and logistical convenience, particularly during initial assembly and pre-stimulation stages, their maturation capacity is limited. Restricted gas exchange within the bag, pH alterations, and insufficient surface adhesion may hinder DC maturation.

This study demonstrates that blood storage bags can be used for preparation, transport, and early stimulation; however, maturation requires a gas- and pH-controlled incubator and an appropriate culture surface. These findings are consistent with the widely accepted "preparation in bag → maturation in culture vessel" workflow used in clinical-scale DC production processes.[10,18,19]

Differences in Antigen Source and Immunogenicity are Important

Variations in maturation responses according to tumor lysate source (more pronounced with MCL lysate and weaker with B-ALL/AML lysates) may be associated with differences in the content of immunostimulatory components, such as DAMPs, neoantigens, nuclear and mitochondrial protein fragments, and heat shock proteins. Indeed, the success of the tumor lysate-loaded DC strategy depends not only on the quantity of antigen but also on the immunogenic characteristics of the cell death pattern underlying lysate formation.[19,21] Therefore, quantitative profiling of markers such as heat shock protein 70/90, high mobility group box 1, and calreticulin in lysates, as well as standardization of immunogenic cell death (ICD) protocols where possible, are recommended for future studies.

The limited increase in CD83 expression observed in the fifth application using cells obtained from a related donor may be consistent with reduced allostimulation due to HLA similarity and/or peripheral immune tolerance mechanisms. This finding suggests that, although DC vaccines primarily aim to achieve antigen specificity, in vitro maturation may partially benefit from allogeneic danger signals. When these signals are weak, the increase in costimulatory molecules may be limited.[10,18,21]

DC-based vaccines are important due to their potential to reduce the risk of relapse, particularly during periods of minimal residual disease or following curative treatments.[18–20] Our workflow combines a low-cost pretreatment phase with a standard 37°C and 5% CO_2 maturation phase, suggesting a feasible approach for centers with limited infrastructure. To demonstrate the clinical value of this approach, further validation is required through (i) functional assays (allo-MLR, IFN-γ ELISPOT, and IL-12p70 release), (ii) evaluation of antigen-specific T-cell activation, and (iii) in vivo safety and response assessments.[10,19,21]

While this study focuses on developing a practical and low-cost experimental approach for dendritic cell production, several factors must be addressed before clinical translation can be considered. In particular, biosafety and quality control considerations are essential for the potential clinical application of this strategy. In clinical-grade dendritic cell production, strict procedures, including sterility testing, endotoxin assessment, standardized antigen preparation, and validated functional assays to confirm antigen-presenting capacity, are required to ensure product safety and reproducibility. These processes are typically performed under GMP conditions using validated quality control assays.[22] Therefore, the present findings should be considered a preliminary proof-of-concept experimental framework that may guide future studies aimed at developing scalable, standardized, and clinically compliant dendritic cell production strategies.

The main limitations of this study include the small sample size and the heterogeneity of the patient population, which consisted of individuals with different hematological malignancies. These factors may limit the generalizability of the findings and should be interpreted with caution. In addition, the study did not include functional assays evaluating the antigen-presenting capacity of the generated dendritic cells, and the potential effects of the pouch material on cytokine or antigen adsorption were not investigated. Future studies should evaluate the production of ICD-standardized lysates and validate antigen/DAMP profiles using techniques such as mass spectrometry or multiplex ELISA. Furthermore, testing alternative culture systems, such as gas-permeable pouches or culture bags, may improve culture efficiency. Optimization of the multiplicity of infection and cell density may increase the consistency of achieving the maturation threshold (≥60%), and the controlled use of maturation enhancers, such as TLR agonists (e.g., poly-I:C or R848), may further enhance dendritic cell maturation.

CONCLUSION

The data suggest that the microenvironment (37°C, 5% CO_2, and surface interaction) is the primary limiting factor for DC maturation, whereas the use of blood bags, although practical during the early phase, is insufficient for maturation. These findings suggest that a low-cost yet biologically accurate production platform can be established and may be translated into clinical applications through functional validation and standardized antigen preparation.

Ethics Committee Approval: Ethics committee approval was obtained from Erciyes University Clinical Research Ethics Committee (Approval Number: 2014/92; Date: 07.02.2014).

Informed Consent: Informed voluntary consent was obtained from all participants.

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