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DEVELOPMENT OF A CAR-T CELL PRODUCT IN A LOCAL LABORATORY SETTING: A STEP TOWARD ACCESSIBLE THERAPY IN KAZAKHSTAN

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Abstract

Introduction. Chimeric Antigen Receptor T-cell (CAR-T) therapy represents a breakthrough in the treatment of relapsed and refractory hematologic malignancies. However, its application is limited by high production costs and technical complexity. In Kazakhstan, approximately 180–240 patients annually require this type of therapy, yet access remains virtually unavailable.

Aim. This study aimed to develop a localized and standardized protocol for the production of CD19-specific CAR-T cells using an automated manufacturing system.

Materials and methods. The CliniMACS Prodigy platform was used for T-cell isolation, activation, and lentiviral transduction with vectors encoding the CD19 CAR construct. Functional activity of CAR-T cells was assessed via flow cytometry using CD19-positive (Raji) and CD19-negative (K562) target cell lines. Quality control included sterility testing, verification of replication-competent lentivirus absence, and phenotypic analysis of the cell product.

Results. The resulting CAR-T cell products met international quality standards in terms of sterility, lack of replication-competent virus, and CD3+CAR+ cell content (75.18%). In vitro experiments demonstrated high specific cytotoxicity against CD19+ targets (Raji) with minimal nonspecific lysis of CD19− cells (K562). The developed and validated protocol enables standardized CAR-T cell manufacturing and has the potential to significantly improve therapy accessibility in Kazakhstan. The data support the feasibility of localized production of effective CAR-T cell products, which could greatly enhance treatment outcomes for patients with severe hematologic malignancies in the region.

Conclusion. The proposed protocol for the production of CD19-specific CAR-T cells using the CliniMACS Prodigy platform meets international standards and allows for the generation of high-quality cellular products for B-cell hematologic cancer therapy.

Keywords: CAR-T cells, CD19, lentiviral vector, T-cell transduction

Introduction. Chimeric Antigen Receptor (CAR-T) Cell-Based Immunotherapy represents a cutting-edge approach in the treatment of hematologic malignancies, particularly B-cell malignancies [1–3].

The core principle of this technology lies in the modification of a patient's T-lymphocytes using viral vectors encoding receptors specific for tumor-associated antigens [4]. These genetically reprogrammed cells gain the ability to effectively recognize and eliminate malignant

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cells expressing the target antigen [5, 6]. In recent years, CAR-T cell therapy has gained widespread application and demonstrated high clinical efficacy; however, issues related to the optimization of production processes and quality control of cell-based products remain highly relevant [7, 8].

CAR-T therapy has been approved for clinical use beyond clinical trials in all developed countries, including North America, the European Union, Southeast Asia, and Australia (Pioneering Biopharma 2021) [9]. Therapeutic products such as Yescarta (for large B-cell lymphoma) and Kymriah (for B-cell acute lymphoblastic leukemia) have been authorized since 2017 [10, 11]. As of now, six CAR-T cell therapy protocols are approved for different indications: Kymriah (Novartis), Yescarta (Gilead), Tecartus (since 2020, Gilead), Breyanzi (since 2021, Bristol-Myers Squibb), Abecma (since 2021, Bristol-Myers Squibb), and Carvykti (since 2022, Janssen Pharma) [12].

The cost of these CAR-T therapeutic products remains extremely high in developed countries, ranging between USD 300,000 and 500,000 per treatment course. For example, Yescarta is priced at USD 373,000 per patient and Kymriah at USD 475,000 per patient [13]. While the cost is justified by their clinical efficacy, it poses a significant barrier. In the final report of the ELIANA clinical trial of Kymriah, five-year follow-up results showed a survival rate of 55% in patients with relapsed or refractory (r/r) B-cell acute lymphoblastic leukemia (ALL) [14]. Notably, 44% of patients remained in remission with no minimal residual disease (MRD), indicating full recovery in these cases [15]. CAR-T technology has revolutionized hematologic oncology by transforming patient outcomes following chemotherapy failure [16].

In Kazakhstan, approximately 7,000 patients with hematologic malignancies are currently registered, with 1,400 new cases diagnosed annually [17]. For patients with relapse following chemotherapy and bone marrow transplantation (BMT), there are currently no effective treatment options available within the country. The limited availability of CAR-T therapy in Kazakhstan and other post-Soviet countries is due not only to its high cost, but also to the complexity of the technology itself [18]. The key stages of CAR-T therapy—lentiviral vector production, CAR-T cell manufacturing, patient preparation, and clinical administration with monitoring (strictly in hospital settings)—are all examples of state-of-the-art, high-tech, knowledge-intensive medicine [19]. As of autumn 2023, Russia had only one officially authorized CAR-T treatment center: the Dmitry Rogachev National Research Center for Pediatric Hematology, Oncology, and Immunology in Moscow [20], with no other clinics offering such therapy. The high treatment cost makes it inaccessible for most Kazakhstani patients to seek CAR-T therapy abroad. Yet, the demand for CAR-T therapy among both patients and oncologists in Kazakhstan is significant. The estimated number of patients in Kazakhstan requiring CAR-T treatment is around 180–240 per year.

Modern platforms for automated cell culture, such as CliniMACS Prodigy, provide standardized CAR-T cell production while minimizing variability and reducing the risk of contamination [21].

Nevertheless, to ensure the efficacy and safety of this therapy, rigorous testing of the cellular product is required, including sterility testing, replication-competent virus (RCV) screening, and evaluation of cytotoxic activity [22].

The present study is aimed at developing and validating protocols for the production of CD19-specific CAR-T cells, including lentiviral transduction of T-lymphocytes, their cultivation, and phenotypic analysis. The study assessed parameters such as transduction efficiency, proliferation, and functional activity of the CAR-T cells, along with thorough quality control of the resulting cell product. The findings of this research will contribute to the improvement of CAR-T cell manufacturing processes and enhance their therapeutic potential.

Materials and Methods.

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Cell Lines, Vectors, and Plasmids

To produce CAR-T cells, HEK293FT cells provided by Kazan Federal University were used, along with the K562 (CD19−) and Raji (CD19+) cell lines obtained from the collection of the National Center for Biotechnology (NCB).

HEK293FT cells were cultured in DMEM supplemented with 10% FBS, L-glutamine, and antibiotics. K562 and Raji cells were maintained in RPMI-1640 medium with similar supplements. Peripheral blood leukocytes obtained by leukapheresis were incubated in TexMACS medium (Miltenyi Biotec) using the CliniMACS Prodigy system.

The CliniMACS Prodigy system is a cell processor designed for the manufacturing of cells used in cell and gene therapy, specifically for the production of Advanced Therapy Medicinal Products (ATMPs) intended for clinical use (Figure 1).

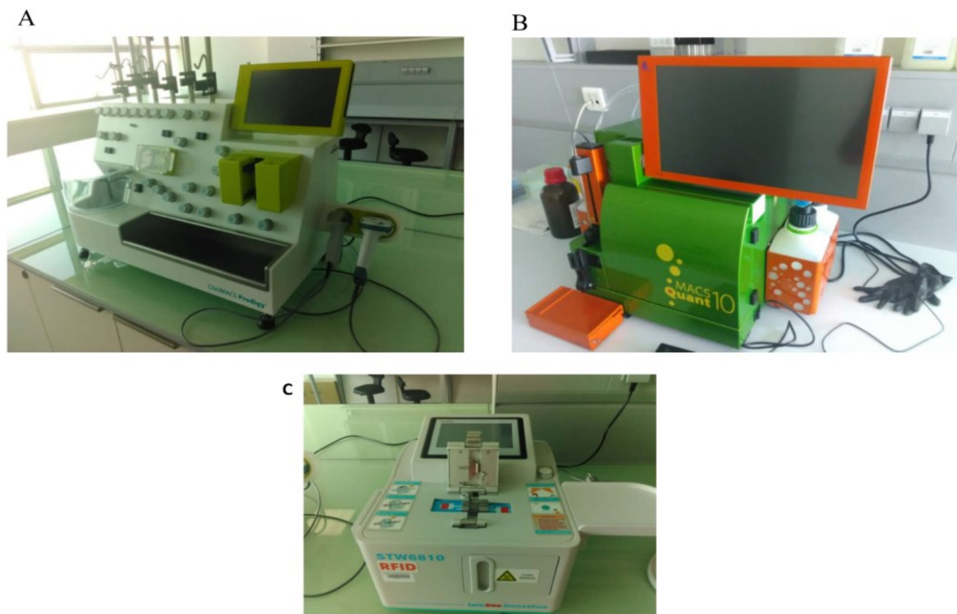

Figure 1. Equipment for the manufacturing of CAR+ cells. A: CliniMACS Prodigy system. B: MACSQuant Analyzer 10 Flow Cytometer (Miltenyi Biotec). C: STW6810 sterile tubing welder (BMS Medicaltech Co.)

Three lentiviral vectors were used for transduction: pHR.CD19_CAR-GFP, pHR.CD19_CAR (developed at the National Center for Biotechnology), and phuCAR (provided by Kazan Federal University). The constructs contain the second-generation CD19 CAR gene (with CD28z or 4-1BB), and in the case of pHR.CD19_CAR-GFP, a GFP marker. Lentiviral vectors were assembled using psPAX2 and pCMV-VSV-G (Addgene).

Lentiviral vector production and titration

HEK293FT cells were transfected with a plasmid mix (pHR.CD19_CAR-GFP, psPAX2, pMD2.G) using branched PEI (Sigma) or Lipofectamine 2000 (ThermoScientific). Culture supernatants containing lentiviral particles were collected at 24, 48, and 72 hours post-transfection.

The functional titer was determined by flow cytometry after transduction of HEK293FT cells with the lentivirus. The percentage of GFP+ cells was measured using the B1 channel (488/(530/30) nm) on the MACSQuant 10 flow cytometer (Miltenyi Biotec). The titer was calculated using the following formula:

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$$\text{Titer}\,(TU/\text{ml}) = \left(\frac{\%GFP+}{100}\right) \times 2 \times 10^5 \times D \times 5$$

CAR-T Cell Production

Leukapheresis samples were obtained at the Scientific and Production Center of Transfusiology (Astana). CD3+ T-lymphocytes were isolated using immunomagnetic separation with the CliniMACS Prodigy system, activated via CD3/CD28 stimulation, and cultured in TexMACS medium supplemented with IL-7 and IL-15.

On Day 1 post-isolation, cells were transduced with a lentiviral vector (MOI = 2). After 12 days, the cells were harvested, analyzed by flow cytometry, and either cryopreserved in HSA + DMSO at –196°C or used for functional testing. A photograph of the CAR+ cell culture taken with the integrated microscope in the system is shown in Figure 2.

Figure 2. Photograph of the CAR+ cell culture in the incubation chamber of the CliniMACS Prodigy system prior to harvest (day 12)

Assessment of Cytotoxic Activity of CAR-T Cells

Cytotoxicity was evaluated in co-culture with Raji ($CD19^+$) and K562 ($CD19^–$) target cells. Target cells were pre-stained with PKH67 (Sigma). Samples were mixed at a ratio of 10:1 (CAR-T:targets), incubated for 24 hours, and the proportion of viable target cells was measured by flow cytometry (B1 channel, MACSQuant 10).

Quality Control of CAR-T Cells

Sterility testing was performed by incubating cell suspensions in 2xYT medium without antibiotics. Mycoplasma contamination was assessed using PCR (Abcam).

A test for replication-competent virus (RCV) was also conducted: five consecutive passages of cell supernatant were performed on HEK293FT cells, followed by PCR analysis for the presence of the POL gene.

Phenotyping of CAR-T cells was performed by analyzing the expression of $CD3^+$, $CD4^+$, $CD8^+$, and CAR^+ markers using monoclonal antibodies and flow cytometry.

Statistical Analysis

Experiments assessing cytolytic activity were conducted in triplicate. The proportions of target cells were compared using Student's t-test. A significance level of $p < 0.05$ was considered indicative of statistically significant differences between mean values.

Results.

Characterization of the Obtained CAR-T Cells

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CAR-T cells were generated using the automated CliniMACS Prodigy platform (Miltenyi Biotec), designed for the production of advanced therapy medicinal products (ATMPs). During the cultivation process on the platform, CD3+ T lymphocytes were separated, stimulated for proliferation, transduced with a lentiviral vector encoding CD19 CAR, and subsequently expanded.

Flow cytometry analysis of the cell product showed that almost all cells in the preparation were T lymphocytes (CD3+), accounting for 99.48% of the total. Among them, 75.18% expressed the CD19 CAR receptor, confirming high transduction efficiency and successful subsequent expansion (Figure 3).

After completion of the cultivation process, CAR+ cells were collected in TexMACS medium. An aliquot of the cell product was used for functional and safety analyses, while the main portion of the cells was cryopreserved in the presence of HSA and DMSO. For detailed phenotypic analysis of the CAR-T cells, staining was performed for CD45+ (leukocytes), CD3+ (T lymphocytes), CD4+ (MHC II-restricted), CD8+ (MHC I-restricted), FMC63 CAR+ (CD19 CAR expression), and cell viability was assessed using propidium iodide.

The CAR-T cell product obtained in this study meets the key quality criteria for cell-based products used in CAR-T therapy.

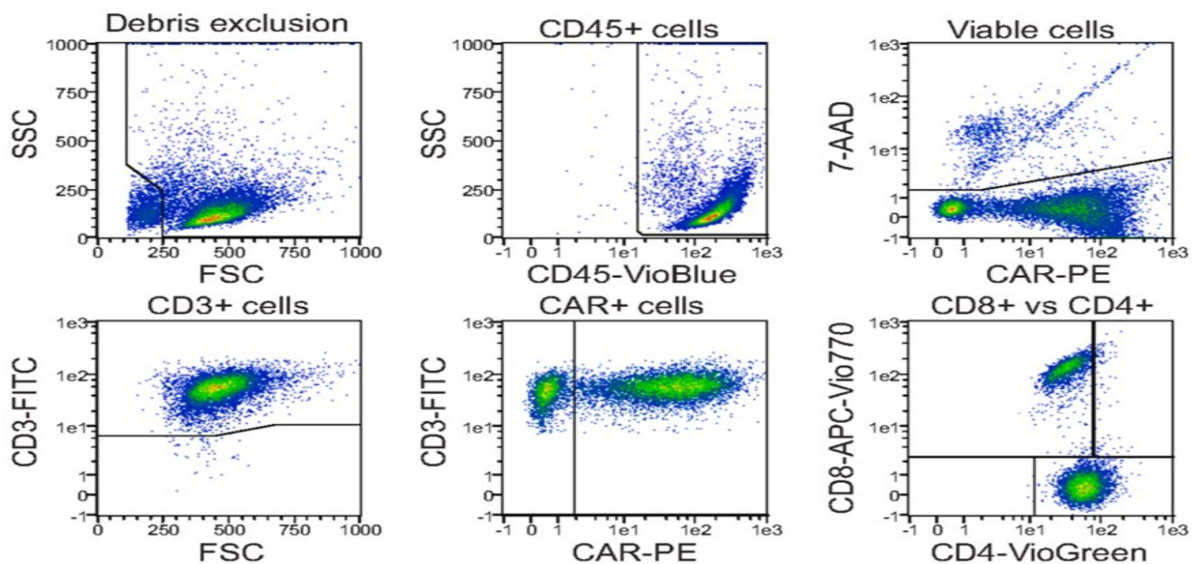

Figure 3. Proportion of CAR+ cells in the cell product obtained using the CliniMACS Prodigy system

Thus, the total number of recorded events was 13,361 (Table 1), reflecting the overall volume of acquired data. Exclusion of cellular debris, based on forward scatter (FSC) parameters, narrowed the dataset to 11,784 events, accounting for 88.2% of all recorded events.

The CD45+ cell population, which includes all viable leukocytes, accounted for 99.67% (11,745 events), among which 90.75% (10,807 events) were viable (CD45+ 7-AAD-), indicating high cell viability after the transduction procedure. Among viable CD3+ T lymphocytes, their proportion was 99.48% (10,604 events), confirming the effectiveness of T-cell isolation prior to the gene modification step.

Analysis of chimeric antigen receptor (CAR) expression showed that CAR+ cells comprised 75.18% of the viable CD3+ cell population (7,972 events), indicating a high efficiency of transduction. Subpopulation analysis of T cells revealed that among CAR+ cells,

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68.94% were CD4+ (5,496 events), 29.85% were CD8+ (2,375 events), and a small fraction (1.22%, 97 events) were CD4-/CD8- cells.

Analysis of the CAR- population among viable CD3+ T lymphocytes showed that their total proportion was 24.82% (2,632 events). Among them, CD4+ cells accounted for 16.83% (1,815 events), CD8+ cells for 30.47% (802 events), and double-positive (CD4+CD8+) and double-negative (CD4-CD8-) cells were represented in significantly smaller amounts — 0.42% (11 events) and 0.15% (4 events), respectively.

Additionally, an analysis was performed on the CD3- cell population, which constituted 0.52% (55 events) of all viable cells. The population of monocytes (CD3- CD14+), isolated from viable CD45+ 7-AAD- cells, was absent in this sample, confirming the successful selection of T lymphocytes.

Table 1. CAR-T Cell Analysis

Cell type	Defined population	Cells/mL	Cells/mL x10	Count	[%]
Sample	all acquired events	1.35E+05	1.35E+05	13361	100
Debris exclusion	FSC small events excluded	1.19E+05	1.19E+05	11784	88.20
CD45+ cells	FSC small events excluded and CD45+	1.19E+05	1.19E+05	11745	99.67
Viable CD45+ cells	CD45+ 7-AAD-	1.08E+05	1.08E+05	10807	90.75
CD3+ cells	Viable CD3+	1.07E+05	1.07E+05	10604	99.48
CAR+ cells	Viable CD3+ CAR+	8.04E+04	8.04E+04	7972	75.18
CAR+ CD4+ cells	Viable CD3+ CAR+ CD8-	5.55E+04	5.55E+04	5496	68.94
CAR+ CD8+ cells	Viable CD3+ CAR+ CD4-	2.38E+04	2.38E+04	2375	29.85
CAR+ CD4- CD8- cells	Viable CD3+ CAR+ CD4- CD8-	9.72E+02	9.72E+02	97	1.22
CAR- cells	Viable CD3+ CAR-	2.66E+04	2.66E+04	2632	24.82
CAR- CD4+ cells	Viable CD3+ CAR- CD8-	1.81E+04	1.81E+04	1815	16.83
CAR- CD8+ cells	Viable CD3+ CAR- CD4-	8.09E+03	8.09E+03	802	30.47
CAR- CD4+ CD8+ cells	Viable CD3+ CAR- CD4+ CD8+	1.12E+02	1.12E+02	11	0.42
CAR- CD4- CD8- cells	Viable CD3+ CAR- CD4- CD8-	4.04E+01	4.04E+01	4	0.15
CD3- cells	Viable CD3-	5.55E+02	5.55E+02	55	0.52
Monocytes	Viable CD3- CD14+ among CD45+ 7-AAD-	0.00E+00	0.00E+00	0	0.00

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Quality Control of the Cell Product

PCR diagnostics for the presence of replication-competent virus (RCV) showed no amplification of specific POL gene fragments, indicating the safety of the cell product. To perform this analysis, serial passaging of the CAR-T cell culture supernatant was conducted using HEK293FT cells, followed by RT-PCR analysis. At all stages of production, no signs of replication-competent lentivirus were detected, confirming compliance of the product with safety requirements.

Additionally, a sterility assessment was carried out by incubating the cell suspension in antibiotic-free 2xYT medium for 7 days, followed by plating on agar Petri dishes. No microbial growth was observed, indicating the sterility of the cell product. Mycoplasma contamination testing was performed using the Mycoplasma PCR Detection Kit (Abcam), and no mycoplasma DNA was detected (Figure 4).

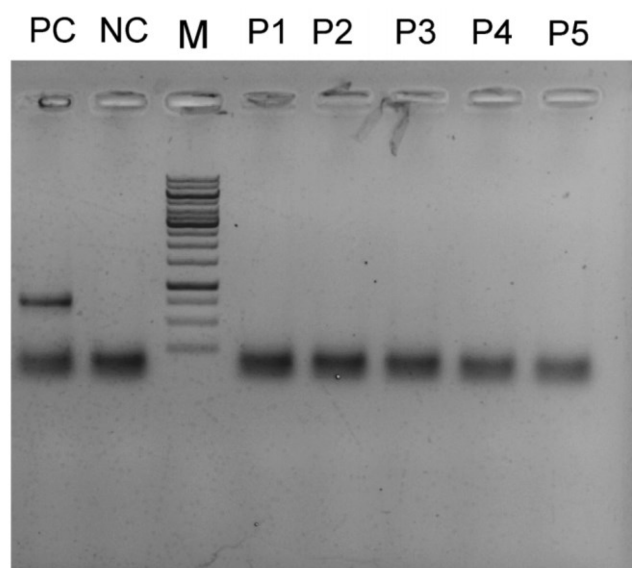

Figure 4. PCR analysis of the cell culture medium for RCV detection.
Lanes: PC – positive control, NC – negative control, P1–P5 – passages of the culture medium

Evaluation of Cytotoxic Activity of CAR-T Cells In Vitro

The cytotoxic activity of the generated CAR-T cells was assessed in co-culture assays with target cells either expressing or lacking the CD19 antigen. The lymphoblastoid cell line Raji ($CD19^+$) was used as the target, and the K562 cell line ($CD19^-$) served as the negative control.

To evaluate target cell lysis, PKH67 fluorescent staining was used, allowing the tracking of the proportion of intact target cells at different time points. Target cells were co-incubated with CAR-T cells at a 10:1 effector-to-target (E:T) ratio, and the percentage of $PKH67^+$ cells was measured by flow cytometry at 0 and 24 hours.

Data analysis showed that after 24 hours of co-culture with CAR-T cells, the number of $CD19^+$ Raji cells decreased by 7.9-fold, indicating strong antigen-specific cytotoxicity of the CAR-T cells toward CD19-expressing targets. In contrast, the number of $CD19^-$ K562 cells decreased only 1.5-fold, confirming a low level of non-specific cytolysis (Figure 5).

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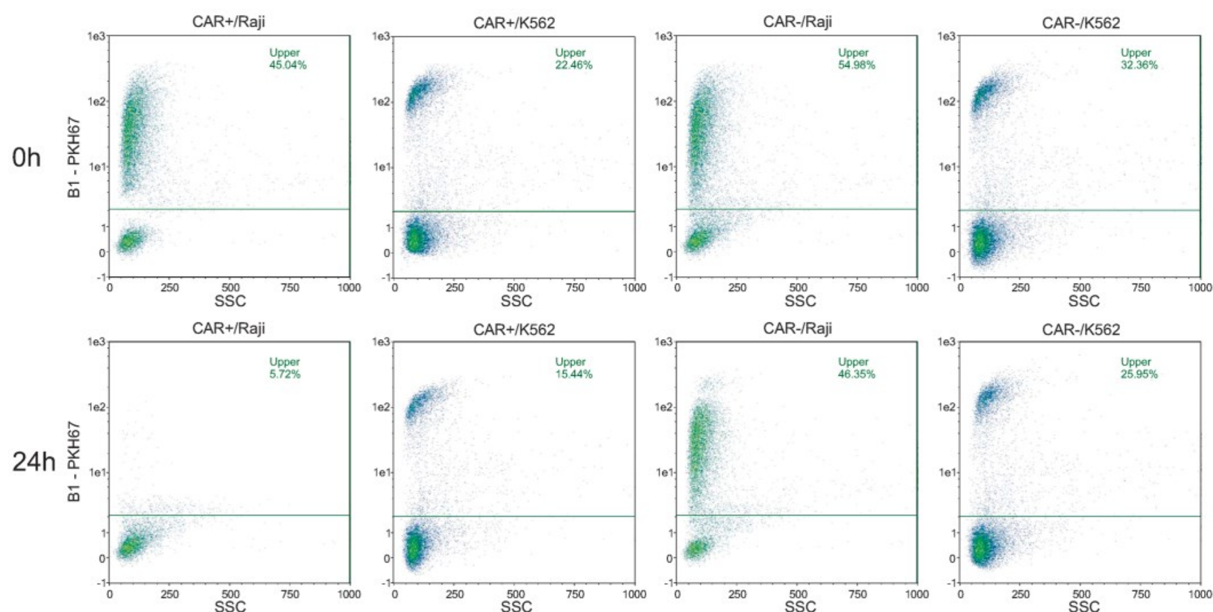

Figure 5. Flow cytometry plots for evaluating the cytolytic activity of CAR-T cells

Generation of Genetic Engineering Materials and Cell Cultures

In the course of this study, a number of lentiviral vectors, plasmids, and cell lines were developed and utilized for the production of CAR-T cells and the assessment of their activity. Table 2 presents a list of the genetic engineering constructs and cell cultures used in the work.

Table 2. Genetically Engineered Materials Created During the Study

No.	Material Name	Description
1	LV CAR s1–s5	Lentiviral particles containing a packaged vector for CD19 CAR receptor transduction
2	pHR.CD19_CAR	Lentiviral vector for expression of the CD19 CAR gene (2nd generation: CD28z/CD3z)
3	pHR.CD19_CAR-GFP	Lentiviral vector carrying CD19 CAR and GFP
4	phuCAR	Lentiviral vector for CD19 CAR expression (obtained from Kazan Federal University)
5	pCD19-2A-Katushka2S	Eukaryotic expression vector carrying CD19 and the fluorescent protein Katushka2S
6	Raji (CD19+)	B-lymphoblastoid cell line used as a target for CAR-T cells
7	K562 (CD19−)	Control erythroleukemia cell line
8	NCI-H522 (CD19+)	Human lung adenocarcinoma cell line expressing CD19
9	PC-3M (CD19+)	Prostate adenocarcinoma cell line expressing CD19
10	MDA-MB-231 (CD19+)	Breast cancer cell line expressing CD19
11	CD19 CAR+ T cells	Human T lymphocytes transduced with a lentiviral vector carrying CD19 CAR

Discussion. The developed protocol for the production of CD19-specific CAR-T cells using the automated CliniMACS Prodigy platform demonstrated high efficiency and compliance with international quality standards. The obtained results reflect not only the high quality of the cellular product but also the practical applicability of the proposed protocol under conditions of limited resources and a previously undeveloped infrastructure for cell therapy in

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the country. Automation of the processes of T-cell isolation, activation, transduction, and expansion enabled the standardization of cell product manufacturing and minimized the risk of contamination, as confirmed by the absence of replication-competent virus and microbial contamination [20].

One of the key indicators of CAR-T cell product quality is transduction efficiency [23]. Flow cytometry analysis of surface marker expression revealed that 75.18% of the obtained cellular product were viable CD3+CAR+ cells, which is consistent with previously reported data on CAR-T cell transduction efficiency [24]. Among them, 68.94% were CD4+ CAR-T cells and 29.85% were CD8+ CAR-T cells, corresponding to the typical distribution of subpopulations in most CAR-T cell products [18].

The results of functional testing showed that the obtained CAR-T cells possess high cytotoxic activity against CD19+ target cells and demonstrate minimal nonspecific lysis of CD19-negative cells (K562). These findings confirm the high specificity and effectiveness of the CD19-CAR-T cells.

One of the main factors influencing the accessibility of CAR-T therapy in Kazakhstan is its high cost [25]. The establishment of local CAR-T cell production may significantly reduce treatment costs by eliminating the need for purchasing commercial products and conducting therapy abroad [26]. A relevant example is the experience of local startups implementing cost-effective yet efficient approaches to CAR-T cell generation with a focus on broader accessibility. Similar initiatives for local CAR-T cell production have proven successful in China and other Asian countries, where in-house cell manufacturing technologies have been developed [27].

The results indicate that the proposed method for standardized CAR-T cell production can be successfully introduced into clinical practice [28]. However, for a full transition to clinical trials, further investigation is needed to understand the factors influencing long-term proliferation and persistence of CAR-T cells in the patient's body [29]. Future research will focus on optimizing the culture medium composition, studying the interaction of CAR-T cells with the tumor microenvironment, and monitoring the long-term efficacy and safety of the therapy [30].

The conducted study confirms the feasibility of local production of CD19-CAR-T cells in Kazakhstan, which may substantially improve the availability of advanced treatment methods for patients with hematologic malignancies.

Strengths of the Study

The main advantage of this study is the successful development and validation of a local protocol for the production of CAR-T cells using the automated CliniMACS Prodigy platform. This approach reduces process variability and minimizes the risk of cellular product contamination, which is particularly important in a region with limited prior experience in cell therapy manufacturing. Another important strength is the rigorous multi-step quality control process, including sterility testing and verification of the absence of replication-competent viruses, ensuring high safety standards for the obtained cell products.

Limitations of the Study

The main limitation of this study is the absence of clinical evaluation of the produced CAR-T cell preparations, as the functional and cytotoxic characteristics were assessed only in vitro. This does not allow for a comprehensive evaluation of potential risks associated with in vivo application, including the possibility of severe side effects such as cytokine release syndrome or neurotoxicity. Another limitation is the small sample size and lack of direct comparison with commercial CAR-T products. It should also be noted that the production of lentiviral vectors requires specialized equipment and highly trained personnel, which may pose a challenge for widespread implementation of this technology in Kazakhstan.

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Conclusion. The obtained results demonstrate that the proposed protocol for the production of CD19-specific CAR-T cells using the CliniMACS Prodigy platform meets international standards and enables the generation of high-quality cell products for the treatment of B-cell hematologic malignancies. The implementation of this technology in Kazakhstan could significantly improve access to effective therapy for patients who previously had no opportunity to receive CAR-T treatment. Further clinical trials are necessary to confirm the therapeutic efficacy and safety of the produced cell products in patients.

Conflict of interest

We declare no conflict of interest.

Authors' contributions

Concept development – Lee S., Shustov A., Tanabaeva Sh., Menlayakova D. Execution - Lee S., Menlayakova D. Processing of results – Tanabaeva Sh., Shustov A., Lee S., D.Gizat, P. Elyasin. Scientific interpretation of the results - Tanabaeva Sh., Lee S., D.Gizat, P. Elyasin. Article writing - Lee S., Shustov A., Tanabaeva Sh., Menlayakova D., D.Gizat, P. Elyasin. This material has not been previously submitted for publication in other publications and is not under consideration by other publishers.

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ЖЕРГІЛІКТІ ЗЕРТХАНА ЖАҒДАЙЫНДА CAR-T ЖАСУШАЛЫҚ ПРЕПАРАТЫН ӘЗІРЛЕУ: ҚАЗАҚСТАНДА ҚОЛЖЕТІМДІ ТЕРАПИЯҒА АДАМ

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Түйіндеме

Кіріспе. Химералық антигендік рецепторлар (CAR-T) негізіндегі терапия – рефрактерлі онкогематологиялық ауруларды емдеудегі серпінді бағыттардың бірі болып табылады. Алайда бұл әдістің қолданылуы өндірістің күрделілігі мен құнының жоғары болуына байланысты шектеулі. Қазақстанда жыл сайын шамамен 180–240 науқас осындай терапияға мұқтаж, бірақ оларға қолжетімділік іс жүзінде жоқ.

Мақсаты. Зерттеу мақсаты — CD19 антигеніне спецификалық CAR-T жасушаларын алу үшін автоматтандырылған жүйені пайдалана отырып, жергілікті және стандартталған өндіріс протоколын әзірлеу.

Материалдар мен әдістер. CD19 CAR рецепторын кодтайтын лентивирустық векторларды пайдалана отырып, Т-лимфоциттерді бөліп алу, белсендіру және трансдукциялау үшін CliniMACS Prodigy автоматтандырылған платформасы қолданылды. CAR-T жасушаларының функционалдық белсенділігі протокеттік цитометрия әдісімен CD19- (K562) және CD19+ (Raji) жасушалық желілерінде зерттелді. Сапаны бақылау стерильділікті, репликацияға қабілетті вирустың болмауын және жасушалық препараттардың фенотиптік сипаттамаларын бағалауды қамтыды.

Нәтижелер. Стерильділік, репликацияға қабілетті вирустың болмауы және CD3+CAR+ жасушаларының үлесі (75,18%) сияқты халықаралық стандарттарға сай келетін CAR-T жасушалық препараттары алынды. *In vitro* жағдайындағы тәжірибелерде CAR-T жасушаларының CD19+ нысан-жасушаларына (Raji) қатысты жоғары спецификалық цитоуыттылығы көрсетілді, ал CD19− жасушаларына қатысты спецификалық емес лизис мардымсыз болды. Жасалған және валидацияланған протокол өндірісті стандарттауға және CAR-T терапиясының Қазақстандағы қолжетімділігін арттыруға мүмкіндік береді. Нәтижелер CAR-T жасушалық препараттарын жергілікті деңгейде тиімді өндіру мүмкіндігін дәлелдейді, бұл өңірдегі ауыр гематологиялық аурулары бар науқастарды емдеу келешегін айтарлықтай жақсарта алады.

Қорытынды. Ұсынылған CliniMACS Prodigy платформасы негізінде CD19-спецификалық CAR-T жасушаларын өндіру протоколы халықаралық талаптарға сәйкес келеді және В-жасушалы онкогематологиялық ауруларды емдеуге арналған жоғары сапалы жасушалық препараттар алуға мүмкіндік береді.

Түйінді сөздер: CAR-T жасушалары, CD19, лентивирустық вектор, Т-лимфоциттер трансдукциясы

СОЗДАНИЕ КЛЕТОЧНОГО ПРЕПАРАТА CAR-T В УСЛОВИЯХ ЛОКАЛЬНОЙ ЛАБОРАТОРИИ: ШАГ К ДОСТУПНОЙ ТЕРАПИИ В КАЗАХСТАНЕ

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Аннотация

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Введение. Терапия на основе химерных антигенных рецепторов (CAR-T) является прорывом в лечении резистентных форм онкогематологических заболеваний, однако её применение ограничено высокой стоимостью и сложностью производства. В Казахстане ежегодно около 180-240 пациентов нуждаются в такой терапии, но доступ к ней практически отсутствует.

Цель. Настоящее исследование посвящено разработке локализованного, стандартизированного протокола производства CAR-T-клеток, специфичных к антигену CD19, с использованием автоматизированной системы.

Материалы и методы. Использовали автоматизированную платформу CliniMACS Prodigy для выделения, активации и трансдукции T-лимфоцитов лентивирусными векторами, кодирующими рецептор CD19 CAR. Оценивали функциональную активность CAR-T-клеток методом проточной цитометрии на клеточных линиях K562 (CD19-) и Raji (CD19+). Контроль качества включал тестирование стерильности, отсутствие репликативно-компетентного вируса и фенотипический анализ клеточных препаратов.

Результаты. Получены CAR-T-клеточные препараты, соответствующие международным стандартам по стерильности, отсутствию репликативно-компетентного вируса и процентному содержанию CD3+CAR+ клеток (75,18%). В опытах in vitro продемонстрирована высокая специфическая цитотоксичность CAR-T-клеток по отношению к CD19+ мишеням (Raji), в то время как неспецифический цитолиз клеток CD19− был незначительным. Разработанный и валидированный протокол позволяет стандартизировать производство и повысить доступность терапии CAR-T в Казахстане. Результаты подтверждают возможность реализации локального производства высокоэффективных CAR-T-препаратов, что может существенно улучшить перспективы лечения пациентов с тяжёлыми формами гематологических заболеваний в регионе.

Заключение. Полученные результаты свидетельствуют о том, что предложенный протокол производства CD19-специфичных CAR-T-клеток с использованием платформы CliniMACS Prodigy соответствует международным критериям и позволяет получать высококачественные клеточные препараты для терапии B-клеточных онкогематологических заболеваний.

Ключевые слова: CAR-T-клетки, CD19, лентивирусный вектор, трансдукция T-лимфоцитов