

Preliminary Report of the Academic CAR-T (ISIKOK-19) Cell Clinical Trial in Turkey: Characterization of Product and Outcomes of Clinical Application

Türkiye Akademik CAR-T Hücre (ISIKOK-19) Klinik Çalışması Ön Raporu: Ürün Karakterizasyonu ve Klinik Uygulama Sonuçları

İ Ebru Erdoğan^{1*}, İ Koray Yalçın^{2,3*}, İ Cansu Hemşinlioğlu², İ Aslıhan Sezgin¹, İ Utku Seyis², İ Derya Dilek Kancağı², İ Cihan Taştan^{2,4}, İ Bulut Yurtsever², İ Raife Dilek Turan², İ Didem Çakırsoy², İ Selen Abanuz², İ Gözde Sır Karakuş², İ Muhammer Elek², İ Hüseyin Saffet Beköz⁵, İ Ali İhsan Gemici⁵, İ Deniz Sargın⁵, İ Mutlu Arat⁶, İ Burhan Ferhanoğlu⁷, İ Ebru Pekgüç⁷, İ Serdar Örnek⁷, İ Deram Büyüktaş⁷, İ Nur Birgen², İ Siret Ratip¹, İ Ercüment Ovalı²

¹Acıbadem Altunizade Hospital, Hematology Unit, İstanbul, Turkey

²Acıbadem Labcell Cellular Therapy Center, İstanbul, Turkey

³Bahçeşehir University Medical Park Göztepe Hospital, Pediatric Bone Marrow Transplantation Unit, İstanbul, Turkey

⁴Üsküdar University, Molecular Biology and Genetics Department of Transgenic Cell Technologies and Epigenetic Application and Research Center (TRGENMER), İstanbul, Turkey

⁵Medipol University Faculty of Medicine, Hematology Unit, İstanbul, Turkey

⁶İstanbul Florence Nightingale Hospital, Hematology Unit, İstanbul, Turkey

⁷VKV American Hospital, Hematology Unit, İstanbul, Turkey

*These authors contributed equally to this work.

Abstract

Objective: Chimeric antigen receptor T (CAR-T) cell therapies have already made an impact on the treatment of B-cell malignancies. Although CAR-T cell therapies are promising, there are concerns about commercial products regarding their affordability and sustainability. In this preliminary study, the results of the first production and clinical data of an academic CAR-T cell (ISIKOK-19) trial in Turkey are presented.

Materials and Methods: A pilot clinical trial (NCT04206943) designed to assess the safety and feasibility of ISIKOK-19 T-cell therapy for patients with relapsed and refractory CD19+ tumors was conducted and participating patients received ISIKOK-19 infusions between October 2019 and July 2021. The production data of the first 8 patients and the clinical outcome of 7 patients who received ISIKOK-19 cell infusions are presented in this study.

Results: Nine patients were enrolled in the trial [5 with acute lymphoblastic leukemia (ALL) and 4 with non-Hodgkin lymphoma (NHL)], but only 7 patients could receive treatment. Two of the 3

Öz

Amaç: Kimerik antijen reseptör T (CAR-T) hücre uygulamaları B-hücreli malignitelerin tedavisinde etkili olmaktadır. CAR-T hücre uygulamalarının sonuçları umut vaadedici olsa da, ticari CAR-T ürünlerinin yüksek maliyetleri nedeniyle ulaşılabilirlik açısından ciddi sorunlar yaşanmaktadır. Bu ön raporda, Türkiye'deki ilk akademik CAR-T hücre çalışmasının üretim ve klinik uygulama sonuçları sunulmuştur.

Gereç ve Yöntemler: Relaps refrakter CD 19+ hematolojik maligniteli hastalarda ISIKOK-19 T-hücre tedavisinin güvenliği ve etkinliğini değerlendirmek amacıyla yürütülen klinik çalışmaya (NCT04206943) Ekim 2019-Temmuz 2021 tarihleri arasındaki hastalar dahil edilmiştir. Bu raporda ilk 8 hastanın üretim bilgileriyle, ISIKOK-19 hücre infüzyonu yapılan 7 hastanın klinik sonuçları sunulmuştur.

Bulgular: Çalışmaya toplam 9 hasta dahil edilmiştir (5 akut lenfoblastik lösemi [ALL] ve 4 non-hodgkin lenfoma [NHL]), ancak sadece 7 hastaya hücre infüzyonu yapılabilmektedir. Hücre infüzyonu alan 3 ALL hastasından 2'sinde ve 4 NHL hastasının 3'ünde tam/kısmi



Abstract

participating ALL patients and 3 of the 4 NHL patients had complete/partial response (overall response rate: 72%). Four patients (57%) had CAR-T-related toxicities (cytokine release syndrome, CAR-T-related encephalopathy syndrome, and pancytopenia). Two patients were unresponsive and had progressive disease following CAR-T therapy. Two patients with partial response had progressive disease during follow-up.

Conclusion: Production efficacy and fulfillment of the criteria of quality control were satisfactory for academic production. Response rates and toxicity profiles were also acceptable for this heavily pretreated/refractory patient group. ISIKOK-19 cells appear to be a safe, economical, and efficient treatment option for CD19+ tumors. However, the findings of this study need to be supported by the currently ongoing ISIKOK-19 clinical trial.

Keywords: Acute lymphoblastic leukemia, Chimeric antigen receptor T (CAR-T) cell, Gene therapy, Non-Hodgkin lymphoma

Öz

cevap gözlenmiştir (toplam yanıt oranı %72). Dört hastada (%57) CAR-T ilişkili toksisite (sitokin salınım sendromu, immün efektör hücre ilişkili nörotoksisite sendromu ve pansitopeni) tespit edilmiştir. İki hastada ise CAR-T hücre uygulaması sonrası cevapsızlık ve progresif hastalık izlenmiştir. Kısmi cevap veren hastalardan 2'sinde de takip sırasında progresif hastalık tespit edilmiştir.

Sonuç: Akademik CAR-T üretimimiz, üretim etkinliği ve kalite kontrol kriterlerinin tam olarak karşılanması açısından tatmin edici sonuçlara sahiptir. Çalışmaya dahil edilen hastaların tedavi yükü hesabına katıldığımda tedaviye cevap oranı ve toksisite profili açısından da sonuçlar kabul edilebilir düzeydedir. Bu sonuçlarla, ISIKOK-19 hücrelerinin güvenli, ekonomik ve etkili bir tedavi seçeneği olduğu düşünülebilir. Ancak bu ön sonuçların halen devam eden ISIKOK-19 klinik çalışmasıyla desteklenmesi beklenmektedir.

Anahtar Sözcükler: Akut lenfoblastik lösemi, Kimerik antijen reseptör T (CAR-T) hücre, Gen terapi, Non-Hodgkin lenfoma

Introduction

Chimeric antigen receptor T cell (CAR-T) therapies have already revolutionized the treatment of B-cell malignancies [1,2]. The commercial availability of CAR-T cell products provides significant real-world experience in treating relapsed or refractory B-cell malignancies, but there are concerns in many countries about commercial products regarding their affordability and sustainability [3,4]. These concerns raised the need for more economical academic and local production. Hence, several countries are exploring alternative T-cell production models that have comparable clinical outcomes to commercial products [5]. ISIKOK-19 is the first local CD19 CAR-T cell product in Turkey, arising from the need to address economical and accessibility concerns related to the use of commercialized products.

Results of the clinical data of the academic CAR-T cell product ISIKOK-19 from Turkey are presented in this preliminary report. Production data for ISIKOK-19 have already been published [6] and are presented as supplementary data in this report.

Materials and Methods

A pilot clinical trial (NCT04206943) designed to assess the safety and feasibility of ISIKOK-19 T-cell therapy in patients with relapsed and refractory CD19+ tumors was conducted at Acbadem Altunizade Hospital. The clinical research protocol was approved by the respective institutional review board and the Blood, Organ, and Tissue Transplantation Department of the Turkish Ministry of Health [(56733164/203)/(2019-11/6)]. All patients provided written informed consent. The participating patients received ISIKOK-19 infusions between October 2019 and July 2021. Production data for the first 8 patients and

the clinical outcomes of 7 patients who received ISIKOK-19 cell infusions are presented in this study. Data on ISIKOK-19 cell production and quality control tests are included in Supplementary Data File 1.

The academic CAR-T cell product presented in this study, ISIKOK-19, encodes the anti-CD19 CAR construct with the single-chain variable fragment (scFv) of an anti-CD19 monoclonal antibody (FMC63) conjugated with the CD8 hinge region, CD28 transmembrane (TM), and co-stimulatory domain, and the CD3 ζ pro-activator signaling domain along with a truncated form of the epidermal growth factor receptor (EGFRt) cell surface protein as a co-expression marker and a safety switch mechanism. Product characterization, in vivo expansion, and persistence of CAR-T cells are presented in Supplementary Data File 2.

CAR-T cell treatment details, eligibility criteria, and primary/secondary outcomes of the study are presented in Supplementary Data File 3.

Results

Nine patients were enrolled in the trial [5 with acute lymphoblastic leukemia (ALL) and 4 with non-Hodgkin lymphoma (NHL)], but only 7 patients could receive treatment (Table 1, Supplementary Data File 4). Three patients with diffuse large B-cell lymphoma (DLBCL), three patients with ALL, and one with double-hit lymphoma (DHL) were treated. One patient had DLBCL transformed from chronic lymphocytic leukemia (CLL) and the patient with DHL had experienced transformation from follicular lymphoma. All four lymphoma patients had refractory disease and two of the ALL patients had extramedullary involvement.

Clinical data illustrate that the study group was very heavily pretreated (median lines of previous therapies = 4), with all except one having a previous history of hematopoietic stem cell transplantation (HSCT), including four patients who underwent allogeneic HSCT. Time from HSCT to ISIKOK-19 infusion was 19,14, 8, and 7 months and donor chimerism at the time of leukapheresis was 100%, 98%, 73%, and 0%, respectively. One of the patients received checkpoint inhibitor therapy (nivolumab) 6 months prior to the ISIKOK-19 infusion. No graft-versus-host disease was observed following the infusion of ISIKOK-19.

Two of the 3 participating ALL patients and 3 of 4 NHL patients had complete/partial response (overall response rate: 72%). All of the patients with DLBCL obtained partial response after infusion of CAR-T cells. All three of the DLBCL patients had bulky disease at the time of CAR-T infusion. The DHL patient had progressive disease following CAR-T cell infusion. Two of the patients with ALL obtained complete response and the other ALL patient was unresponsive to treatment. Four patients (57%) had CAR-T-related toxicities [cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), and pancytopenia] (Table 2).

Table 1. Patient characteristics.

Patient	Age/gender	Disease	Pre-CAR-T treatments	Pre-CAR-T LDH/Ferritin/CRP	HSCT before CAR-T	Bridging therapy	CAR-T dose (total)	Response	Toxicity
1	52/M	DLBCL, bulky	RT, CHOP, R-Hyper-CVAD, ibrutinib	177/1536/8.9	Allogenic (DLI+) Post-HSCT Time: 19 months	RT	5x10 ⁶ /kg	PR	CRES Grade 1
2	22/F	ALL	ALL-IC BFM 2009, RT FLAG-IDA	207/2376/3.3	Allogenic Post-HSCT Time: 14 months	No	6.4x10 ⁶ /kg	CR	CRS Grade 2
3	31/F	ALL	Hyper-CVAD	138/1541/0.19	Allogenic Post-HSCT Time: 8 months	Vincristine Methylprednisolone	4.5x10 ⁶ /kg	PD	No
4	57/F	DHL	R-Bendamustine, ESHAP, GEMOX	13/596/1.1	Autologous	RT	4.8x10 ⁶ /kg	PD	No
5	39/M	DLBCL, bulky	R-EPOCH-R, R-ICE, RT, Nivolumab, GDP	86/2453/5.7	Autologous	No	4x10 ⁶ /kg	PR	CRS Grade 1
6	54/M	DLBCL bulky	R-CHOP, ICE	596/600/16.8	No	No	7.3x10 ⁶ /kg	PR	No
7	20/M	ALL	Hyper-CVAD, FLAG-IDA	260/1555/2.6	Allogenic Post-HSCT Time: 7 months	No	4x10 ⁶ /kg	CR	CRS Grade 2 CRES Grade 1

CAR-T: Chimeric antigen receptor T; M: male; F: female; DLBCL: diffuse large B-cell lymphoma; ALL: acute lymphoblastic leukemia; DHL: double-hit lymphoma; RT: radiotherapy; LDH: lactate dehydrogenase; CRP: C-reactive protein; HSCT: hematopoietic stem cell transplantation; DLI: donor lymphocyte infusion; CRES: CAR-T-related encephalopathy syndrome; CRS: cytokine release syndrome; PR: partial response; CR: complete response; PD: progressive disease.

Table 2. Clinical outcomes according to diagnosis.

Diagnosis	Number of patients	Number of treated patients	Number of bridging therapies	ORR	CR	PR	NR	Toxicity
ALL	5	3	1	67%	2 (67%)	-	1 (33%)	2 CRS (67%), 1 CRES (33%)
NHL	4	4	2	75%	-	3 (75%)	1 (25%)	1 CRS (25%), 1 CRES (25%)

ORR: Overall response rate; CR: complete response; PR: partial response; NR: no response; ALL: acute lymphoblastic leukemia; NHL: non-Hodgkin lymphoma; CRS: cytokine release syndrome; CRES: CAR-T-related encephalopathy syndrome.

Infusion of ISIKOK-19 was associated with mild and transient toxicity; one patient had grade 1 CRS and another one had grade 1 ICANS, both of which resolved without intervention. One patient with grade 2 CRS needed a single dose of tocilizumab and recovered afterwards. One patient had grade 2 CRS, grade 1 ICANS, and hematological toxicity with pancytopenia. He required both tocilizumab and dexamethasone treatment for recovery. The hematological toxicity resolved by the end of the second month of treatment. All four patients who had CRS or ICANS were responsive to ISIKOK-19 therapy. All patients with the exception of one experienced B-cell aplasia following ISIKOK-19 infusion. B-cell aplasia lasted longer in responding patients.

Discussion

In this study, 8 peripheral mononuclear cell aphereses were performed for 8 patients. One production attempt failed and the patient died just before CAR-T cell infusion. Seven of the eight production attempts resulted in successful outcomes that met all the criteria for release and quality control. The products also had the advantage of cost-effectiveness as previously demonstrated by Ran et al. [7], who reported the low cost of decentralized CAR-T cell production in an academic nonprofit setting.

Four of 7 patients in this study had a history of allogeneic HSCT prior to CAR-T cell infusion. Three of these 4 patients had high ratios of donor chimerism (100%, 98%, and 73%) at the time of leukapheresis and one of them was non-chimeric. The patients with high donor chimerism showed responses to CAR-T cell treatment, whereas the non-chimeric patient was unresponsive. This finding is in accordance with previous CAR-T cell reports from patients with high donor chimerism [8]. We speculate that the satisfactory results in cases with high donor chimerism may have been due to the non-exhausted T-cells of donors in comparison to the probably exhausted T-cells of heavily pretreated patients. This finding is encouraging for CAR-T cell treatments from allogeneic sources.

Overall, the toxicity observed in this study was mild. Grade 2 CRS was noted in 2 patients who required intervention. All responders had adverse events, whereas non-responders had no toxicity. This finding is supportive of observations regarding a positive correlation between adverse events and the efficacy of CAR-T cell therapy in previous reports [9,10]. Although a high tumor burden is associated with more adverse events in CAR-T cell therapy [11], it has been illustrated that fractionated dosing with intra-patient dose modification optimizes safety without compromising efficacy [12]. The low toxicity rates in this study, which mainly included patients with high tumor burdens, can be explained by the dose administration being split over 3 days.

Regarding the in vivo expansion of CAR-T cells, study data indicate higher vector copy numbers for responders. Lasting high CAR-T copy numbers and vanishing CD19 positivity in responsive patients were evidence of the efficacy of ISIKOK-19 cells. In the responsive lymphoma patients, biopsy specimens following disease progression showed that progression occurred in the CD19- lymphoma cell population. This finding is in accordance with previously reported high rates of CD19 negativity in relapses that mostly occurred within the first 4 months following CAR-T cell therapy [13].

Study Limitations

One limitation of this study was the inclusion of mainly heavily pretreated refractory patients. This influenced the clinical outcome in this small group of patients. Furthermore, the COVID-19 pandemic led to the death of one of the study patients. The findings of this study need to be supported by the current ongoing larger ISIKOK-19 clinical trial.

Conclusion

This report has presented the production and clinical outcomes of the first academic CAR-T cell trial in Turkey. Production efficacy and the criteria of quality control were fulfilled for academic production. Response rates and toxicity profiles were acceptable for this heavily pretreated/refractory patient group. ISIKOK-19 cells appear to be a safe, economical, and effective treatment option for CD19+ tumors.

Ethics

Ethics Committee Approval: Acıbadem Mehmet Ali Aydınlar University, number: 2019-11/6.

Authorship Contributions

Data Collection or Processing: E.E., K.Y., C.H., A.S., U.S., D.D.K., C.T., B.Y., R.D.T., D.Ç., S.A., G.S.K, M.E., H.S.B., A.İ.G., D.S., M.A., B.F., E.P., S.Ö., D.B., N.B., S.R., E.O.; Analysis or Interpretation: K.Y., E.O.; Writing: K.Y., E.E., S.R., E.O.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: All funding of this work was supported by the Technology and Innovation Funding Programs Directorate (TEYDEB) of the Scientific and Technological Research Council of Turkey (TÜBİTAK) and the Acıbadem Healthcare Group.

References

1. Mullard A. FDA approves first CAR T therapy. *Nature Rev Drug Discov* 2017;16:669.
2. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, Braunschweig I, Oluwole OO, Siddiqi T, Lin Y, Timmerman JM, Stiff PJ, Friedberg JW, Flinn IW, Goy A, Hill BT, Smith MR, Deol A, Farooq U,

McSweeney P, Munoz J, Avivi I, Castro JE, Westin JR, Chavez JC, Ghobadi A, Komanduri KV, Levy R, Jacobsen ED, Witzig TE, Reagan P, Bot A, Rossi J, Navale L, Jiang Y, Aycock J, Elias M, Chang D, Wiezorek J, Go WY. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med* 2017;377:2531-2544.

3. Neelapu SS, Dickinson M, Ulrickson ML, Oluwole OO, Herrera AF, Thieblemont C, Ujjani CS, Lin Y, Riedell PA, Kekre N, de Vos S, Yang Y, Milletti F, Goyal L, Kawashima J, Chavez JC. Interim analysis of ZUMA-12: a phase 2 study of axicabtagene ciloleucel (Axi-Cel) as first-line therapy in patients (pts) with high-risk large B cell lymphoma (LBCL). *Blood* 2020;136(Suppl 1):49.

4. Shah NN, Hamadani M. Is there still a role for allogeneic transplantation in the management of lymphoma? *J Clin Oncol* 2021;39:487-498.

5. Castella M, Caballero-Baños M, Ortiz-Maldonado V, González-Navarro EA, Suñé G, Antoñana-Vidósola A, Boronat A, Marzal B, Millán L, Martín-Antonio B, Cid J, Lozano M, García E, Tabera J, Trias E, Perpiña U, Canals JM, Baumann T, Benítez-Ribas D, Campo E, Yagüe J, Urbano-Ispizua Á, Rives S, Delgado J, Juan M. Point-of-care CAR T-cell production (ARI-0001) using a closed semi-automatic bioreactor: experience from an academic phase I clinical trial. *Front Immunol* 2020;11:482.

6. Taştan C, Kaňçağı DD, Turan RD, Yurtsever B, Çakırsoy D, Abanuz S, Yılanıcı M, Seyis U, Özer S, Mert S, Kayhan CK, Tokat F, Açıkkel Elmas M, Birdoğan S, Arbak S, Yalçın K, Sezgin A, Kızılkılıç E, Hemşinlioğlu C, İnce Ü, Ratip S, Ovalı E. Preclinical assessment of efficacy and safety analysis of CAR-T cells (ISIKOK-19) targeting CD19-expressing B-cells for the first Turkish academic clinical trial with relapsed/refractory ALL and NHL patients. *Turk J Hematol* 2020;37:234-247.

7. Ran T, Eichmuller SB, Schmidt P, Schlander M. Cost of decentralized CAR T-cell production in an academic nonprofit setting. *Int J Cancer* 2020;147:3438-3445.

8. Brudno JN, Somerville RP, Shi V, Rose JJ, Halverson DC, Fowler DH, Gea-Banacloche JC, Pavletic SZ, Hickstein DD, Lu TL, Feldman SA, Iwamoto AT, Kurlander R, Maric I, Goy A, Hansen BG, Wilder JS, Blacklock-Schuber B, Hakim FT, Rosenberg SA, Gress RE, Kochenderfer JN. Allogeneic T cells that express an anti-CD19 chimeric antigen receptor induce remissions of B-cell malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease. *J Clin Oncol* 2016;34:1112-1121.

9. Park JH, Rivière I, Gonen M, Wang X, Sénéchal B, Curran KJ, Sauter C, Wang Y, Santomaso B, Mead E, Roshal M, Maslak P, Davila M, Brentjens RJ, Sadelain M. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N Engl J Med* 2018;378:449-459.

10. Nastoupil LJ, Jain MD, Spiegel JY, Ghobadi A, Lin Y, Dahiya S, Lunning MA, Lekakis LJ, Reagan PM, Oluwole OO, McGuirk JP, Deol A, Sehgal AR, Goy A, Hill BT, Andreadis C, Munoz J, Westin JR, Chavez JC, Cashen AF, Bennani NN, Rapoport AP, Vose JM, Miklos DB, Neelapu SS, Locke FL. Axicabtagene ciloleucel (Axi-Cel) CD19 chimeric antigen receptor (CAR) T-cell therapy for relapsed/refractory large B-cell lymphoma: real world experience. *Blood* 2018;132(Suppl 1):91.

11. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, Fry TJ, Orentas R, Sabatino M, Shah NN, Steinberg SM, Stroncek D, Tschernia N, Yuan C, Zhang H, Zhang L, Rosenberg SA, Wayne AS, Mackall CL. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet* 2015;385:517-528.

12. Frey NV, Shaw PA, Hexner EO, Pequignot E, Gill S, Luger SM, Mangan JK, Loren AW, Perl AE, Maude SL, Grupp SA, Shah NN, Gilmore J, Lacey SF, Melenhorst JJ, Levine BL, June CH, Porter DL. Optimizing chimeric antigen receptor T-cell therapy for adults with acute lymphoblastic leukemia. *J Clin Oncol* 2020;38:415-422.

13. Vercellino L, Di Blasi R, Kanoun S, Tessoulin B, Rossi C, D'Aveni-Piney M, Obéric L, Bodet-Milin C, Bories P, Olivier P, Lafon I, Berriolo-Riedinger A, Galli E, Bernard S, Rubio MT, Bossard C, Meignin V, Merlet P, Feugier P, Le Gouill S, Ysebaert L, Casasnovas O, Meignan M, Chevret S, Thieblemont C. Predictive factors of early progression after CAR T-cell therapy in relapsed/refractory diffuse large B-cell lymphoma. *Blood Adv* 2020;4:5607-5615.

Supplementary Data Files
Supplementary Data File 1. https://cms.galenos.com.tr/SolvePark/Uploads/Files/supplementary-data-39-3-s1.pdf
Supplementary Data File 2. https://cms.galenos.com.tr/SolvePark/Uploads/Files/supplementary-data-39-3-s2.pdf
Supplementary Data File 3. https://cms.galenos.com.tr/SolvePark/Uploads/Files/supplementary-data-39-3-s3.pdf
Supplementary Data File 4. https://cms.galenos.com.tr/SolvePark/Uploads/Files/supplementary-data-39-3-s4.pdf