haematologica
Journal of the Ferrata Storti Foundation

A rescue approach in refractory diffuse large B-cell lymphoma with obinutuzumab-redirected cytokine-induced killer cells: a first-in-human case report

by Francesca Elice, Roberta Sommaggio, Elisa Cappuzzello, Marcello Riva, Maria Chiara Tisi, Martina Bernardi, Angela Bozza, Daniela Catanzaro, Katia Chieregato, Anna Merlo, Ilaria Giarretta, Anna Dalla Pietà, Mauro Krampera, Carlo Visco, Livio Trentin, Andrea Visentin, Alberto Tosetto, Giuseppe Astori, and Antonio Rosato

Received: January 19, 2024.
Accepted: April 29, 2024.

Citation: Francesca Elice, Roberta Sommaggio, Elisa Cappuzzello, Marcello Riva, Maria Chiara Tisi, Martina Bernardi, Angela Bozza, Daniela Catanzaro, Katia Chieregato, Anna Merlo, Ilaria Giarretta, Anna Dalla Pietà, Mauro Krampera, Carlo Visco, Livio Trentin, Andrea Visentin, Alberto Tosetto, Giuseppe Astori, and Antonio Rosato. A rescue approach in refractory diffuse large B-cell lymphoma with obinutuzumab-redirected cytokine-induced killer cells: a first-in-human case report. Haematologica. 2024 May 9. doi: 10.3324/haematol.2023.284881 [Epub ahead of print]

A rescue approach in refractory diffuse large B-cell lymphoma with obinutuzumab-redirected cytokine-induced killer cells: a first-in-human case report

Running Title: **Obinutuzumab-retargeted CIK cells for R/R DLBCL**

Francesca Elice[1,*], Roberta Sommaggio[2,3,*], Elisa Cappuzzello[3], Marcello Riva[1], Maria Chiara Tisi[1], Martina Bernardi[4], Angela Bozza[4], Daniela Catanzaro[4], Katia Chieregato[4], Anna Merlo[4], Ilaria Giaretta[1], Anna Dalla Pietà[2], Mauro Krampera[5], Carlo Visco[5], Livio Trentin[6], Andrea Visentin[6], Alberto Tosetto[1], Giuseppe Astori[4,#], Antonio Rosato[2,3,#]

1. Hematology, San Bortolo Hospital, Vicenza, Italy
2. Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy
3. Veneto Institute of Oncology-IOV IRCCS, Padua, Italy
4. Advanced Cellular Therapy Laboratory, Hematology, San Bortolo Hospital, Vicenza, Italy
5. Hematology and Bone Marrow Transplant Unit, Section of Biomedicine of Innovation, Department of Engineering for Innovative Medicine, University of Verona, Verona, Italy
6. Hematology Unit, Department of medicine, University of Padua, Padua, Italy

* Francesca Elice and Roberta Sommaggio contributed equally to the work as principal investigator

[#] Giuseppe Astori and Antonio Rosato contributed equally as senior authors

Corresponding author: Elisa Cappuzzello (elisa.cappuzzello@iov.veneto.it)

Funding This study was supported by AIRC Foundation - IG 2018 - ID. 2135; AIRC Foundation under 5x1000 2019 - ID. 22759 program Veneto Institute of Oncology IOV - I.R.C.C.S -5x1000 fund; Ricerca Corrente 2024 to AR; AVILL-AIL - Associazione Italiana Leucemie, Associazione Vicentina per le Leucemie e i Linfomi - Bando Benvenuti.

Data-sharing statement Data used in this study will be provided to qualified researches on reasonable request.

Contributions FE and RS contributed equally to the work as principal investigator, and AR and GA contributed equally as senior authors. RS and AR designed preclinical experiments; EC, ADP, RS performed preclinical experiments and interpreted the data; FE and MR designed clinical research; FE, MR, MK, MCT, LT, CV, AV and AT discussed the clinical research; MB, AB, DC, KC, AM and GA developed and produced CIK_D332 under GMP conditions; GI performed CAR-T monitoring; FE, AR, RS, EC and GA wrote the manuscript; FE, AR, RS and GA conceived and directed the project. All authors critically reviewed and approved the final manuscript.

Disclosures None.

The therapeutic scenario for relapsed/refractory (R/R) aggressive B cell lymphomas has been impressively revolutionized in the last few years by the advent of chimeric antigen receptor T cell (CAR-T) therapy. This approach represents a substantial advancement for this difficult-to-treat population, with a perspective of potential cure in approximately 40% of cases (1, 2). Unfortunately, a substantial fraction of patients cannot access this complex treatment due to several main reasons: most regulatory agencies require stringent inclusion criteria that substantially limit the eligibility of patients; the production of effective CAR-T cells requires functional T lymphocytes, leading to occasional failure in the engineering procedure, especially in heavily pre-treated patients, or recently exposed to cytotoxic agents; only few specialized centers are authorized for CAR-T treatment, in relation to the expertise required to manage its specific complications, e.g. cytokine release syndrome (CRS), neurological toxicity, and prolonged B cell aplasia (3). Furthermore, the CAR-T cell production process is laborious and expensive due to technical and safety obstacles associated with the genetic modification of T cells.

Many of these obstacles could be overcome if cytotoxic effector cells are easily generated in clinically relevant numbers, and are endowed with tumor specificity without genetic modification. In this regard, a promising approach already explored in several clinical studies involves cytokine-induced killer (CIK) cells (www.cik-info.org), with applications spanning various tumor subtypes including lymphoblastic leukemia and aggressive lymphomas (4). CIK cells can be derived and expanded from peripheral blood mononuclear cells (PBMCs) (5, 6), and exhibit phenotypic and functional properties akin to both NK and T cells, such as CD3 and CD56 antigens expression. Their MHC-unrestricted antitumor activity has been exploited across a wide range of tumor histotypes without requiring prior antigen exposure or specific priming. The lytic and antitumor activity of CIK cells mainly relies on the recruitment and activation of NKG2D (7, 8). Extensively explored in preclinical and clinical settings (as reviewed in (3)), CIK cell-based immunotherapy proved feasible and remarkably low toxic. Specifically, in 85 clinical trials that enrolled 4,039 patients, CIK cell-based immunotherapy demonstrated high tolerability with only mild side effects, such as fever, chills, fatigue, headache, and skin rashes (4). Noteworthy, allogeneic CIK cells are almost completely devoid of graft-versus-host disease (GvHD) effects. CIK cells can be produced for any patient starting from small volumes of peripheral blood (PB, 25-30 ml), even when the $CD3^+$ T lymphocyte count is extremely low and the tumor burden is high (5, 9, 10). This is made possible thanks to a culture protocol using the bispecific mAb blinatumomab, which fosters the generation of relevant numbers of CIK cells, leading to the complete eradication of the potentially contaminant neoplastic component within the culture (9).

CIK cells can represent a very interesting tool for adoptive cell immunotherapy (ACT) also for their relevant expression of CD16 (FcgRIIIa) (11). Indeed, the combination with clinical-grade monoclonal antibodies (mAb) allows CIK cell activity to be redirected in an antigen-specific manner, leading to antibody-dependent cell-mediated cytotoxicity (ADCC) against both solid tumors (11, 12) and B-cell malignancies (9). In the latter neoplastic context, CIK cells exert a more relevant and significant cytotoxic activity against both B cell

lines and autologous neoplastic targets when combined with the anti-CD20 mAb obinutuzumab (9) (Figure 1).

The compelling results generated so far both in vitro and in animal models provide robust support to the hypothesis that the combination of an easily *ex vivo* expandable population of immune effector cells, such as CIK cells, with clinical-grade mAbs, may represent an effective and feasible therapeutic strategy.

We tested this approach for the first time in December 2022, when we treated a 59-year-old woman with a diffuse large B-cell lymphoma (DLBCL) relapsed after four lines of therapy including CAR-T cell therapy, and concomitant severe cytopenia, the latter feature limiting other therapeutic options. Specifically, the patient presented in January 2021 with a stage IVB DLBCL of non-germinal center subtype, and triple expressor status (BCL2, BCL6 and MYC), but without genetic rearrangement. Clinically, she had nodal and splenic involvement, fever and PB cytopenia without evidence of lymphoma in the bone marrow (bone marrow biopsy and immunophenotype resulted negative). Comorbidities included adrenal insufficiency after adenoma resection in glucocorticoid replacement treatment, and undifferentiated collagenopathy. The patient underwent one course of R-CHOP (rituximab, cyclophosphamide, vincristine, doxorubicin and prednisone) and five courses of R-DA-EPOCH (rituximab plus dose-adjusted etoposide, vincristine, doxorubicin, prednisone and cyclophosphamide), and obtained a complete response. Seven months later, however, she experienced lymphoma progression that was histologically confirmed in a nodal biopsy. Upon obtaining the informed consent from the patient, PB was collected at that time, and CIK cells were produced and cryopreserved for potential subsequent administration. She was then treated with two cycles of R-DHAOX (rituximab, dexamethasone, cytarabine and oxaliplatin), with evidence of a partial response, and underwent collection of PB stem cells with the aim to proceed to autologous stem cell transplant. However, despite two additional cycles of R-DHAOX, the disease progressed again, and CAR-T cell therapy was planned. Apheresis for lymphocyte collection was performed in June 2022 and bridge therapy consisted of dexamethasone and two cycles of rituximab-polatuzumab and bendamustine, with complete remission at the PET/CT scan but persistent fever and cytopenia. Production of CAR-T cell tisagenlecleucel resulted in an out of specification (OOS) product because of low interferon-gamma release in the final quality test. Nevertheless, the patient was infused in August 2022 after lymphodepletion with fludarabine and cyclophosphamide. Only transient fever (CRS grade 1) was observed after OOS tisagenlecleucel infusion, but neutropenia grade 3 and thrombocytopenia grade 2 occurred and persisted over months. The expansion of OOS CAR-T cells was limited and delayed, with a peak concentration of 14 cells/µL at day +21, and small numbers of circulating cells (1-2.5 CAR-T cells/µL) persisting at different time points until sixteen months after infusion. PET/CT scans carried out at three months post-infusion revealed lymphoma progression in a cervical node (increased SUV from 1.9 to 5.9 in a previous site of lymphoma, Deauville score 4), and new metabolic uptake in few small mediastinal lymph nodes (Deauville score 3). Thus, we proceeded to the infusion of CIK cells (CIK_D332) and obinutuzumab treatment, according to the Hospital

Exemption rule as per Regulation EC 1394/2007, after receiving the approval from the local Ethical Committee and the national drug authority (AIFA, Agenzia Italiana del Farmaco), in addition to the consent of the patient.

CIK_D332 production had been performed in this case 14 months earlier, starting from a small volume of PB (26 mL) without any apheresis procedure. Twenty-five million PBMCs were isolated by density gradient centrifugation that yielded a total of $732x10^6$ CIK_D332 cells after 14 days of culture under GMP conditions, using the blinatumomab mAb-based protocol described in the study by Dalla Pietà *et al.* (9, and Figure 2A). CIK_D332 final product had a viability of 93.59%, as measured as the percentage of 7-amino actinomycin D negative cells, and was composed of $CD3^+CD56^+$ (59.24%), $CD3^+CD56^-$ (40.43%), $CD3^-CD56^+$ (0.18%), $CD3^-CD56^-$ (0.15%), $CD19^+$ (0.01%), and $CD20^+$ (0.03%) cells (Figure 2B). Lytic activity of CIK_D332 was assessed using a calcein-AM release assay against K562 (human chronic myelogenous leukemia) cell line at different effector/target (E/T) ratios. Cytotoxicity was 61%, 41%, 13% and 2% at E/T ratios of 40:1, 20:1, 4:1 and 1:1, respectively (Figure 2C). The final product was frozen in the vapor phase of liquid nitrogen. The patient was infused at a 14-day interval with four escalating doses of CIK_D332, from $1x10^6$ to $3x10^6$ cells/kg. The day before each infusion, the patient received obinutuzumab (1000 mg) (Figure 2D).

Patient follow-up revealed the absence of noteworthy inflammation, neurotoxicity or CRS, as shown by the lack of significant cytokine release in the plasma (Figure 3A-B). The sole treatment-emergent adverse effect was persistent neutropenia and transient reduction in platelets (grade 4) after the administration of obinutuzumab (Figure 3C). Thrombocytopenia, however, can be accounted for the obinutuzumab administration, as reported in the drug information leaflet.

In terms of efficacy, the PET scan performed one month after the fourth and last CIK infusion demonstrated the stability of the cervical node but the disappearance of metabolic activity in the small mediastinal nodes (Figure 3D-E). The PET scan carried out four months after the end of the therapy showed a reduction of the dimension of the cervical node (diameter from 13 to 8 mm with calcifications) without any other hypermetabolic site, clinically indicating a very good partial remission. At the time of this report, the lymphoma is still stable 12 months after the first cell infusion without evidence of progression, thus showing that CIK_D332 treatment durably halted disease progression without severe treatment-related events.

In conclusion, this 59-year-old woman with a chemo-resistant R/R DLBCL achieved a remarkable clinical response upon a therapeutic approach that combines CIK cells and a CD20 antigen-retargeting mAb. This result highlights the therapeutic potential of CIK cell-based strategies as a promising and feasible alternative for patients facing relapse, refractoriness, or ineligibility for standard therapies, even after CAR-T cell therapy failure. The potential for widespread applicability and the favorable safety profile pave the way to a new treatment paradigm for CD20-positive non-Hodgkin lymphoma patients.

References

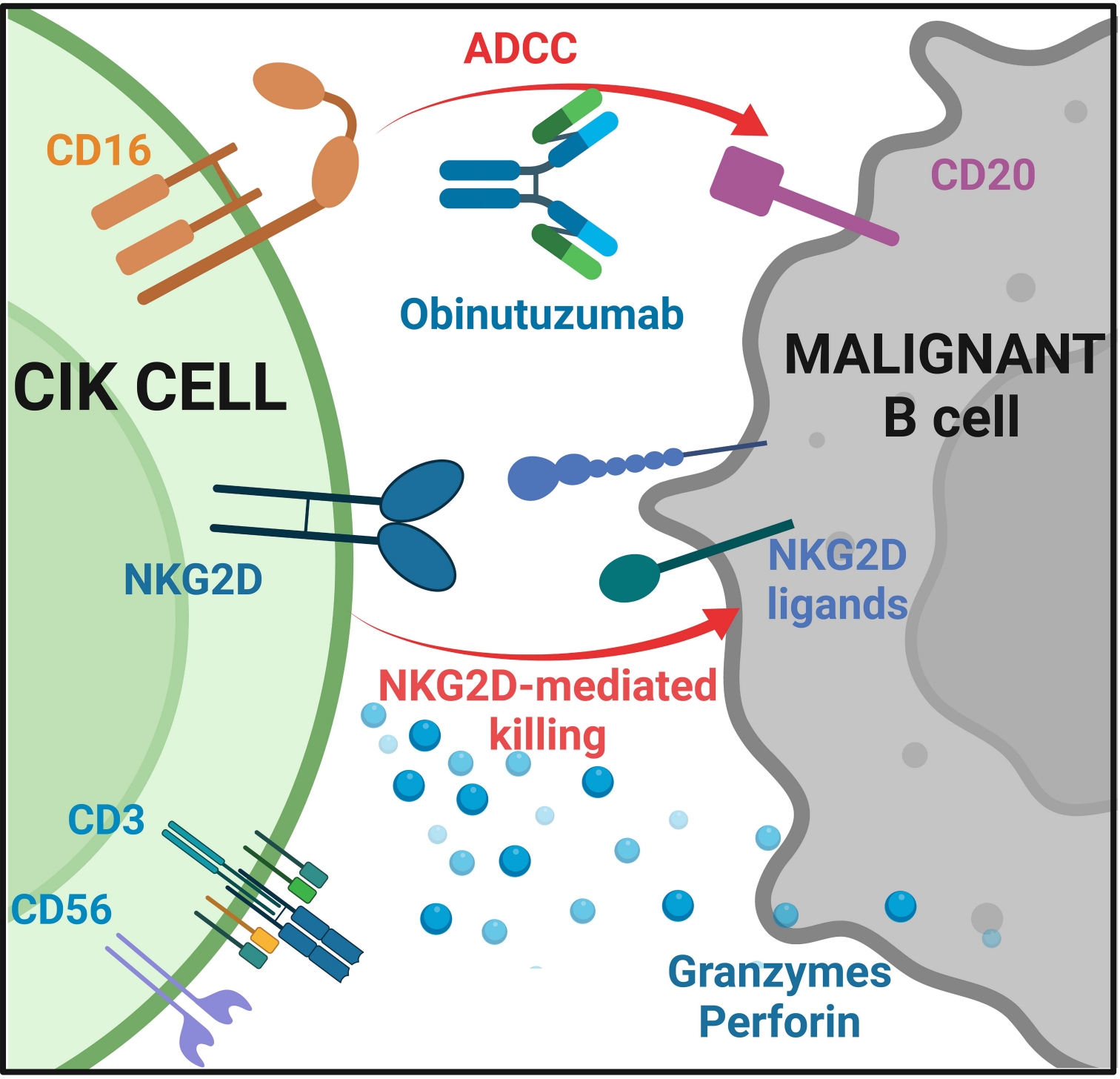
1. Sermer D, Batlevi C, Palomba ML, et al. Outcomes in patients with DLBCL treated with commercial CAR T cells compared with alternate therapies. Blood Adv. 2020;4(19):4669-4678.
2. Cai Q, Zhang M, Li Z. Potential strategies against resistance to CAR T-cell therapy in haematological malignancies. Ther Adv Med Oncol. 2020;12:1758835920962963.
3. Cappuzzello E, Vigolo E, D'Accardio G, et al. How can Cytokine-induced killer cells overcome CAR-T cell limits. Front Immunol. 2023;14:1229540.
4. Zhang Y, Schmidt-Wolf IGH. Ten-year update of the international registry on cytokine-induced killer cells in cancer immunotherapy. J Cell Physiol. 2020;235(12):9291-9303.
5. Palmerini P, Dalla Pietà A, Sommaggio R, et al. A serum-free protocol for the ex vivo expansion of Cytokine-Induced Killer cells using gas-permeable static culture flasks. Cytotherapy. 2020;22(9):511-518.
6. Schmidt-Wolf IG, Negrin RS, Kiem HP, et al. Use of a SCID mouse/human lymphoma model to evaluate cytokine-induced killer cells with potent antitumor cell activity. J Exp Med. 1991;174(1):139-149.
7. Pievani A, Borleri G, Pende D, et al. Dual-functional capability of CD3+CD56+ CIK cells, a T-cell subset that acquires NK function and retains TCR-mediated specific cytotoxicity. Blood. 2011;118(12):3301-3310.
8. Verneris MR, Karimi M, Baker J, et al. Role of NKG2D signaling in the cytotoxicity of activated and expanded CD8+ T cells. Blood. 2004;103(8):3065-3072.
9. Dalla Pietà A, Cappuzzello E, Palmerini P, et al. Innovative therapeutic strategy for B-cell malignancies that combines obinutuzumab and cytokine-induced killer cells. J Immunother Cancer. 2021;9(7):e002475.
10. Lu XC, Yang B, Yu RL, et al. Clinical study of autologous cytokine-induced killer cells for the treatment of elderly patients with diffuse large B-cell lymphoma. Cell Biochem Biophys. 2012;62(1):257-265.
11. Cappuzzello E, Tosi A, Zanovello P, et al. Retargeting cytokine-induced killer cell activity by CD16 engagement with clinical-grade antibodies. Oncoimmunology. 2016;5(8):e1199311.
12. Sommaggio R, Cappuzzello E, Dalla Pietà A, et al. Adoptive cell therapy of triple negative breast cancer with redirected cytokine-induced killer cells. Oncoimmunology. 2020;9(1):1777046.

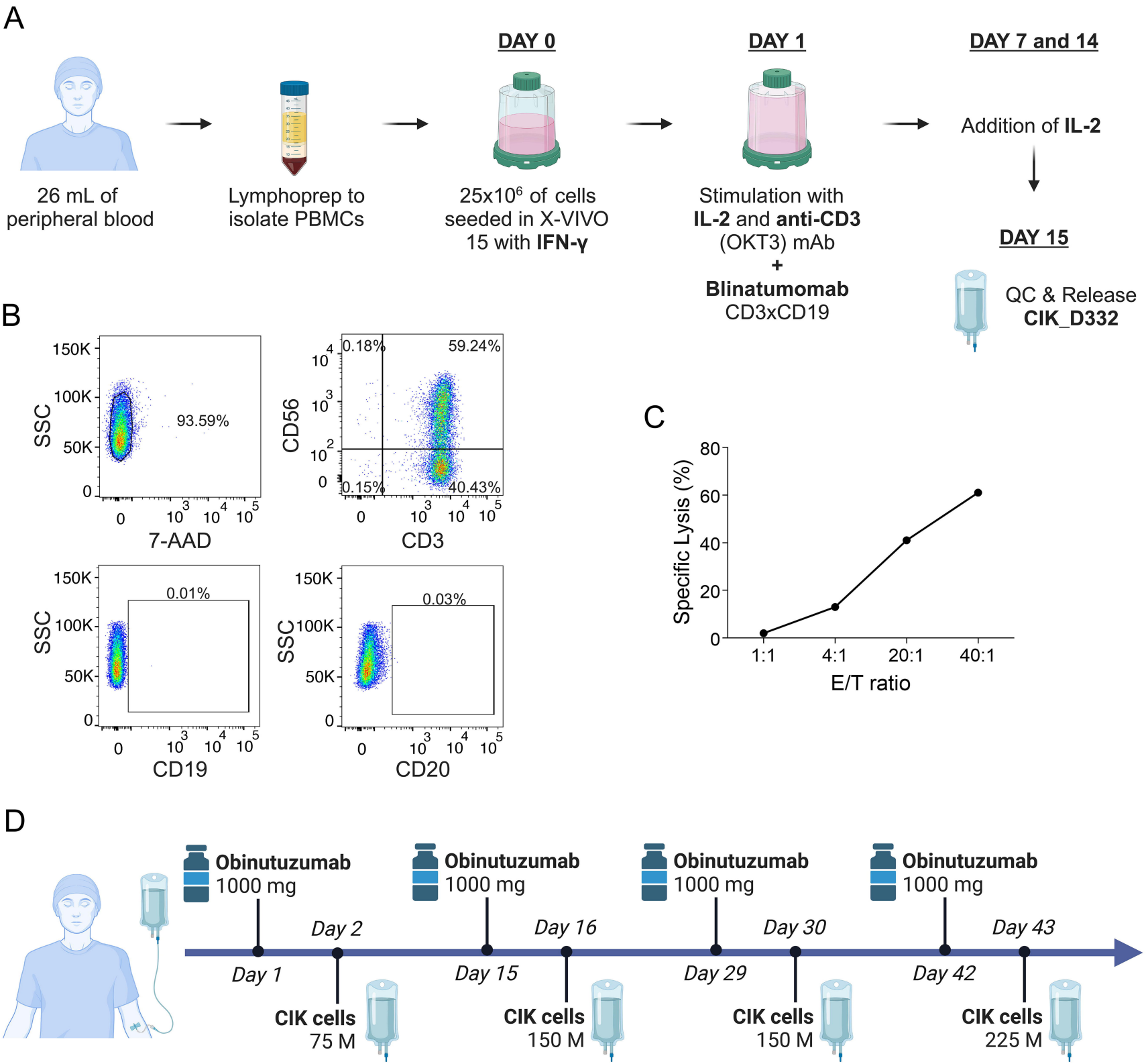
Figure legends

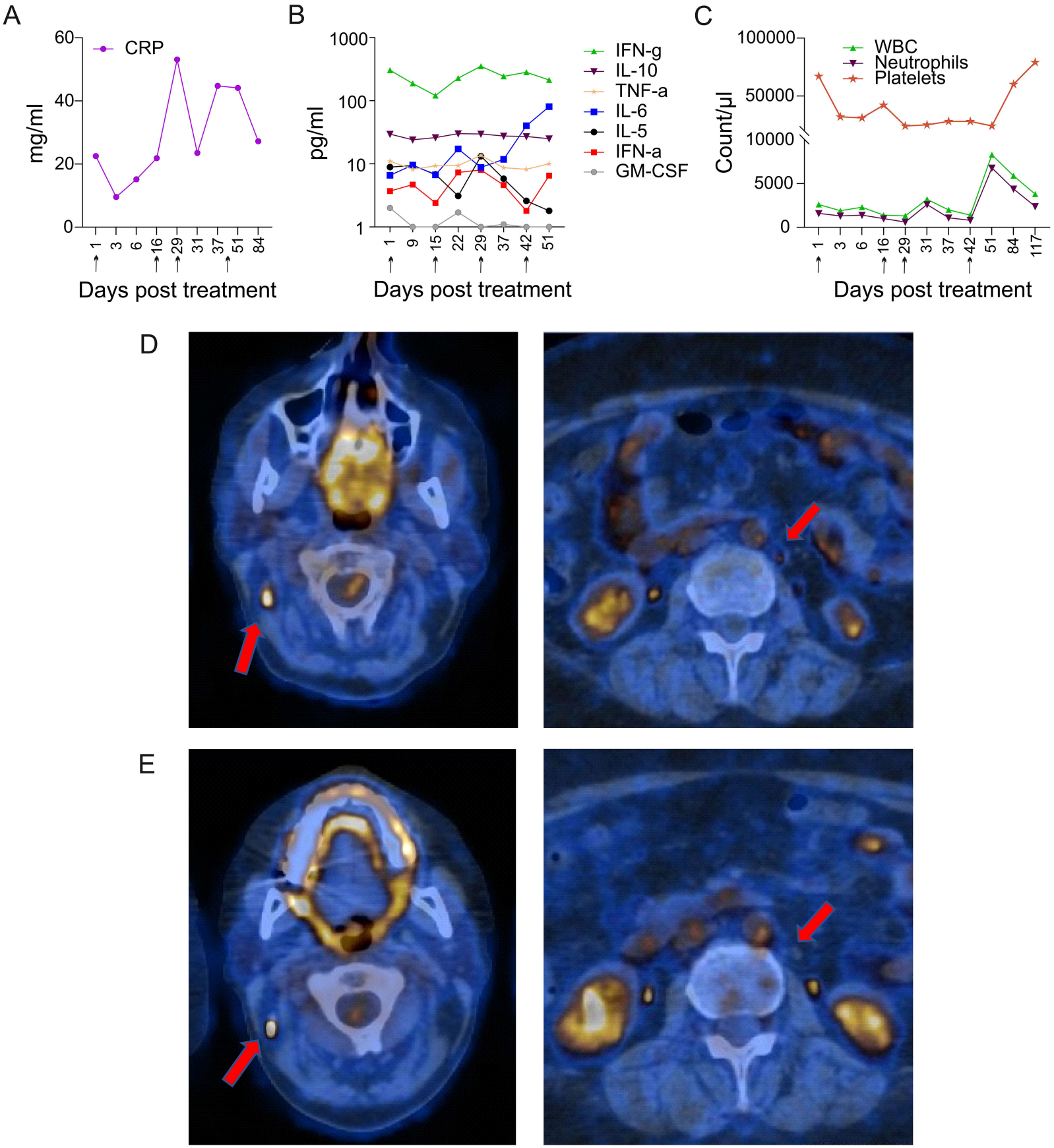
Figure 1: Mab-mediated CIK cell retargeting. The combination of Cytokine-Induced Killer (CIK) cells with clinical-grade anti-CD20 mAbs, such as Obinutuzumab, polarizes their activity in an antigen-specific manner and prompts ADCC. Created with BioRender.com.

Figure 2: CIK_D332 production and infusion protocol. (A) CIK_D332 were generated using a blinatumomab mAb-based cell culture protocol in the presence of IFN-γ, CD3 (OKT3) mAb, and IL-2 for 14 days in G-Rex flasks (Wilson Wolf, Saint Paul, MN). The infusion product was evaluated prior to cryopreservation by (B) multi-color flow cytometry to characterize the phenotype and by (C) calcein-AM release assay against K562 cell line to determine the cytotoxic activity at different effector/target (E/T) ratios. (D) The patient was infused at a 14-day interval with escalating doses of CIK_D332 from $1x10^6$ to $3x10^6$ cells/kg. The day before each infusion, the patient received the administration of the anti-CD20 mAb obinutuzumab (1000 mg). Created with BioRender.com.

Figure 3: Clinical markers after CIK_D332 infusion. Post-infusion trends of serum C-reactive protein (CRP) (A), plasma cytokines (B), and WBCs, neutrophils and platelets (C). Arrows indicate the administration of the combined CIK cells and obinutuzumab treatments, as detailed in Figure 2D. PET scans were performed (D) before and (E) one month after the treatment. Arrows indicate the involved lymph nodes.


ADCC
CD16
CD20
Obinutuzumab
CIK CELL
MALIGNANT
B cell
NKG2D
NKG2D
ligands
NKG2D-mediated
killing
CD3
CD56
Granzymes
Perforin


A
DAY 0
DAY 1
DAY 7 and 14
26 mL of peripheral blood
Lymphoprep to isolate PBMCs
25x10^6 of cells seeded in X-VIVO 15 with IFN-γ
Stimulation with IL-2 and anti-CD3 (OKT3) mAb + Blinatumomab CD3xCD19
Addition of IL-2
DAY 15
QC & Release CIK_D332
B
SSC
7-AAD
93.59%
CD56
CD3
0.18%
59.24%
0.15%
40.43%
CD19
0.01%
CD20
0.03%
C
Specific Lysis (%)
E/T ratio
1:1
4:1
20:1
40:1
D
Obinutuzumab 1000 mg
Day 1
Day 2
Day 15
Day 16
Day 29
Day 30
Day 42
Day 43
CIK cells 75 M
CIK cells 150 M
CIK cells 150 M
CIK cells 225 M


A
CRP
mg/ml
60
40
20
0
1
3
6
16
29
31
37
51
84
Days post treatment
B
IFN-g
IL-10
TNF-a
IL-6
IL-5
IFN-a
GM-CSF
pg/ml
1000
100
10
1
1
9
15
22
29
37
42
51
Days post treatment
C
WBC
Neutrophils
Platelets
Count/µl
100000
50000
10000
5000
0
1
3
6
16
29
31
37
42
51
84
117
Days post treatment
D
E