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

The therapeutic scenario for relapsed/refractory aggressive B-cell lymphomas has been impressively revolutionized in the last few years by the advent of chimeric antigen receptor (CAR) T-cell 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 for several major 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 pretreated patients, or those recently exposed to cytotoxic agents; and only a few specialized centers are authorized to provide CAR T-cell treatment, in relation to the expertise necessary to manage its specific complications, e.g. cytokine release syndrome, 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 could be easily generated in clinically relevant numbers, and could be 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,[5,6] and exhibit phenotypic and functional properties akin to those of both natural killer and T cells, such as CD3 and CD56 antigen 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 relies mainly on the recruitment and activation of NKG2D.[7,8] Extensively explored in preclinical and clinical settings,[3] CIK cell-based immunotherapy has proven feasible and of remarkably low toxicity. 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] It is worth noting that allogeneic CIK cells are almost completely devoid of graft-*versus*-host disease effects. CIK cells can be produced for any patient starting from small volumes of peripheral blood (25-30 mL), even when the $CD3^+$ T lymphocyte count is extremely low and the tumor burden is high.[5,9,10] This is possible thanks to a culture protocol using the bispecific monoclonal antibody 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 also because of their relevant expression of CD16 (FcγRIIIa).[11] Indeed, combination with clinical-grade monoclonal antibodies allows CIK cell activity to be redirected in an antigen-specific manner, leading to antibody-dependent cell-mediated cytotoxicity 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 monoclonal antibody 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 monoclonal antibodies, 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 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 diffuse large B-cell lymphoma 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 peripheral blood cytopenia without evidence of lymphoma in the bone marrow (bone marrow biopsy and immunophenotype resulted negative). Comorbidities included adrenal insufficiency after adenoma resection being treated with glucocorticoid replacement, and undifferentiated collagenopathy. The patient received one course of R-CHOP (rituximab, cyclophosphamide, vincristine, doxorubicin and prednisone) and five courses of R-DA-EPOCH (rituximab plus

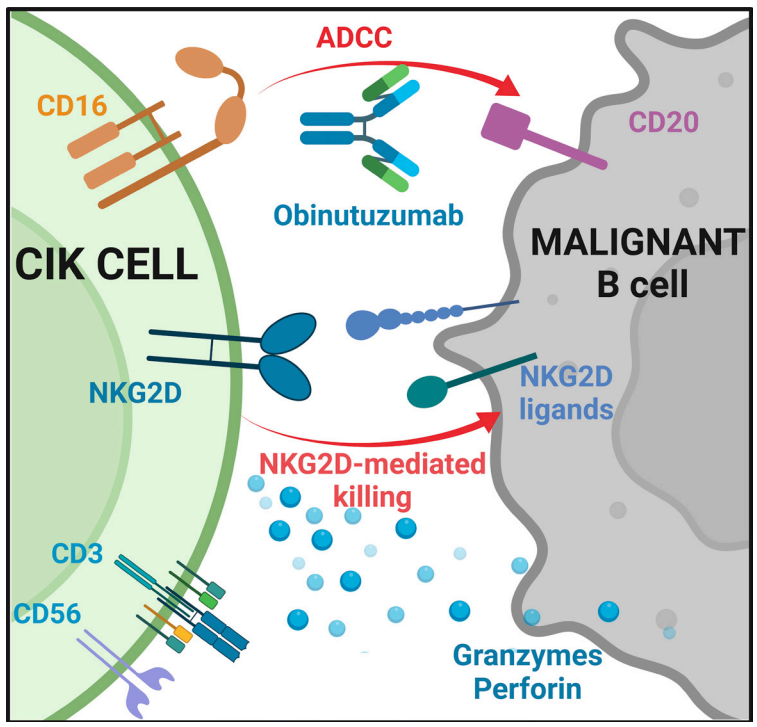
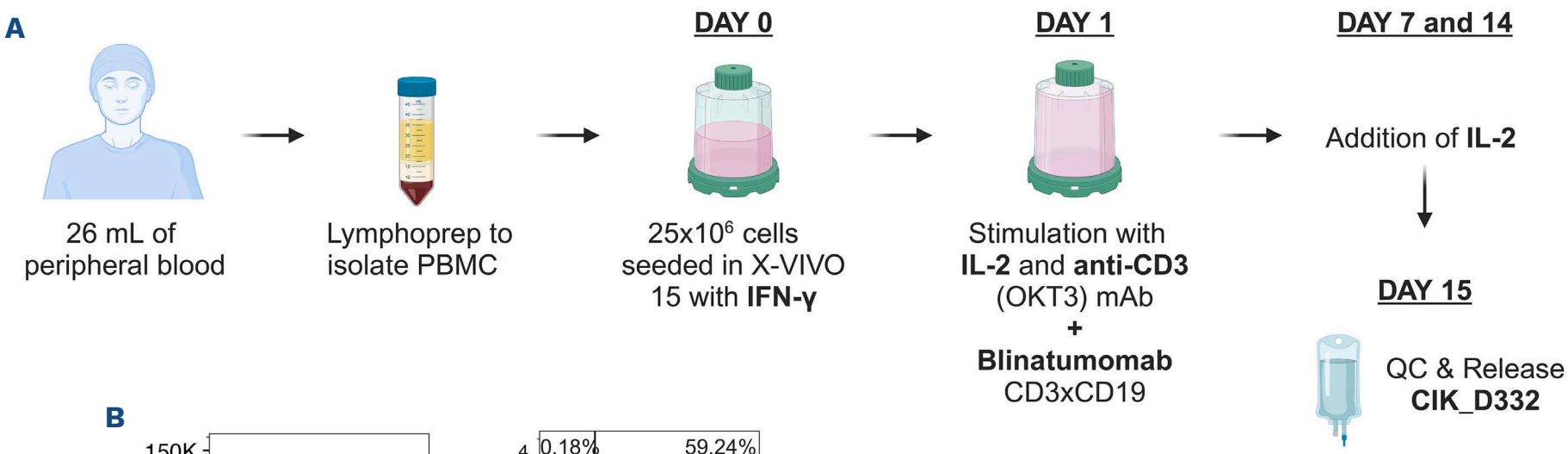


Figure 1. Monoclonal antibody-mediated cytokine-induced killer cell retargeting. The combination of cytokine-induced killer cells with clinical-grade anti-CD20 monoclonal antibodies, such as obinutuzumab, polarizes their activity in an antigen-specific manner and prompts antibody-dependent cell-mediated cytotoxicity. Figure created with BioRender.com. CIK: cytokine-induced killer; NKG2D: natural killer group 2 D; ADCC: antibody-dependent cell-mediated cytotoxicity.

dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin), and obtained a complete response. Seven months later, however, she experienced lymphoma progression that was histologically confirmed in a nodal biopsy. Upon obtaining informed consent from the patient, peripheral blood 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 peripheral blood 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 bridging therapy consisted of dexamethasone and two cycles of rituximab-polatuzumab and bendamustine, with complete remission as determined by positron emission tomography and computed tomography (PET/CT) scans, but persistent fever and cytopenia. Production of CAR T-cell tisagenlecleucel resulted in an out-of-specification product because of low interferon-γ release in the final quality test. Nevertheless, the patient was infused in August 2022 after lymphodepletion with fludarabine and cyclophosphamide. Only transient fever (cytokine release syndrome grade 1) was observed after infusion of the tisagenlecleucel, but neutropenia grade 3 and thrombocytopenia grade 2 occurred and persisted over months. The expansion of 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 timepoints until 16 months after the infusion. PET/CT scans carried out 3 months after the infusion revealed lymphoma progression in a cervical node (an increase in the standardized uptake value from 1.9 to 5.9 in a previous site of lymphoma, Deauville score 4), and new metabolic uptake in a few small mediastinal lymph nodes (Deauville score 3). Thus, we proceeded to infusion of the 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 had been produced in this case 14 months earlier, starting from a small volume of peripheral blood (26 mL) without any apheresis procedure. Twenty-five million peripheral blood mononuclear cells were isolated by density gradient centrifugation, which yielded a total of $732x10^6$ CIK_D332 cells after 14 days of culture under Good Manufacturing Practice conditions, using the blinatumomab-based protocol described in the study by Dalla Pietà *et al.*[9] (Figure 2A). The 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). The 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 14-day intervals with four escalating doses of CIK_D332, from $1x10^6$ to $3x10^6$ cells/kg. The day before each infusion, the patient received obinutuzumab (1,000 mg) (Figure 2D). The patient's follow-up revealed the absence of noteworthy inflammation, neurotoxicity or cytokine release syndrome, 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 a transient reduction in platelets (grade 4) after the administration of obinutuzumab (Figure 3C). The thrombocytopenia, however, could be explained by the obinutuzumab administration, as reported in the drug information leaflet.

In terms of efficacy, the PET scan performed 1 month after the fourth and last CIK infusion demonstrated the stability of the cervical node disease but the disappearance of metabolic activity in the small mediastinal nodes (Figure 3D, E). The PET scan carried out 4 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,



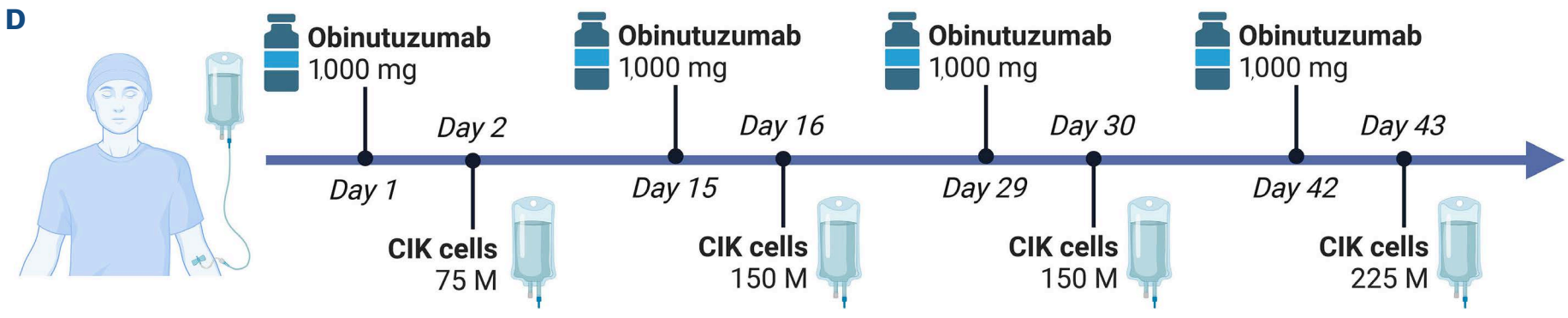


Figure 2. CIK_D332 production and infusion protocol. (A) CIK_D332 were generated using a blinatumomab-based cell culture protocol in the presence of interferon-γ, CD3 (OKT3) monoclonal antibody, and interleukin-2 for 14 days in G-Rex flasks (Wilson Wolf, Saint Paul, MN, USA). The infusion product was evaluated prior to cryopreservation by (B) multi-color flow cytometry to characterize the phenotype and by (C) a calcein-AM release assay against the K562 cell line to determine the cytotoxic activity at different effector-to-target cell ratios. (D) The patient was infused at 14-day intervals with escalating doses of CIK_D332 from $1x10^6$ to $3x10^6$ cells/kg. The day before each infusion, the patient was given the anti-CD20 monoclonal antibody obinutuzumab (1,000 mg). Figure created with BioRender.com. PBMC: peripheral blood mononuclear cells; IFN-γ: interferon-gamma; IL-2: interleukin-2; mAB: monoclonal antibody; QC: quality control; SSC: side scatter; 7-AAD: 7-amino actinomycin D; E:T: effector-to-target cell ratio; CIK: cytokine-induced killer.

clinically indicating a very good partial remission. At the time of writing 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 chemoresistant relapsed/refractory diffuse large B-cell lymphoma achieved a remarkable clinical response to a therapeutic approach that combines CIK cells and a CD20 antigen-retargeting monoclonal antibody. 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 ther-

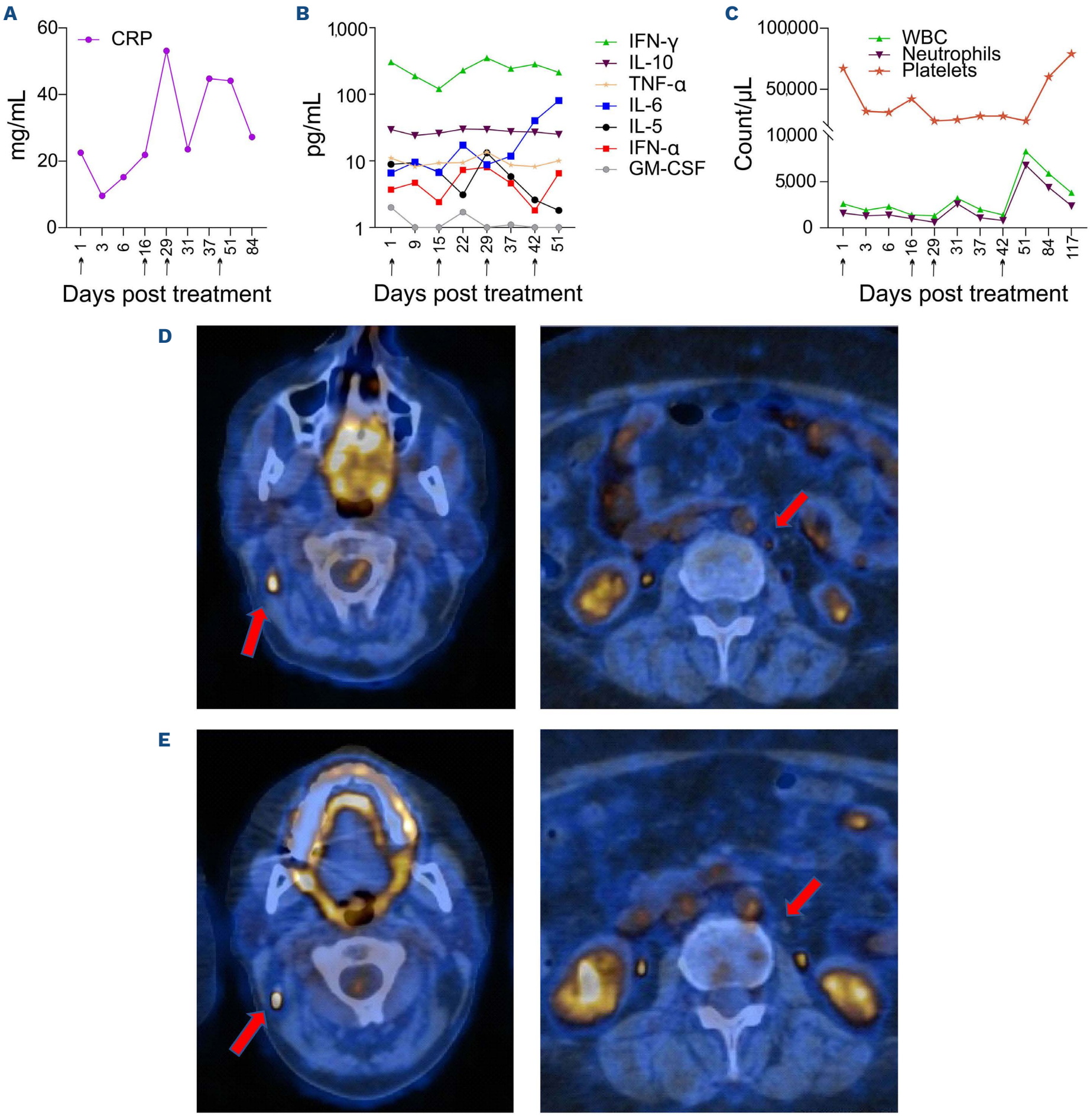


Figure 3. Clinical markers after CIK_D332 infusion. (A-C) Post-infusion trends of serum C-reactive protein (A), plasma cytokines (B), and white blood cells, neutrophils and platelets (C). Arrows indicate the times of administration of the combined cytokine-induced killer cell and obinutuzumab treatments, as detailed in Figure 2D. (D, E) Positron emission tomography scans were performed before (D) and 1 month after (E) the treatment. Arrows indicate the involved lymph nodes. CRP: C-reactive protein; IFN-γ: interferon-gamma; IL: interleukin; TNF-α: tumor necrosis factor-alpha; IFN-α: interferon-alpha; GM-CSF: granulocyte-monocyte – colony-stimulating factor; WBC: white blood cells.

apies, even after the failure of CAR T-cell therapy. The potential for widespread applicability and the favorable safety profile pave the way to a new treatment paradigm for patients with CD20-positive non-Hodgkin lymphoma.

Authors

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#] and Antonio Rosato[2,3#]

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

*FE and RS contributed equally as first authors.
#GA and AR contributed equally as senior authors.

Correspondence:
E. CAPPUZZELLO - elisa.cappuzzello@iov.veneto.it

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Disclosures

No conflicts of interest to disclose.

Contributions

FE and RS contributed equally to the work as principal investigators, and AR and GA contributed equally as senior authors. RS and AR designed the preclinical experiments. EC, ADP, and RS performed the preclinical experiments and interpreted the data. FE and MR designed the 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 Good Manufacturing Practice conditions. GI performed the CAR T-cell 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.

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Data-sharing statement

Data used in this study will be provided to qualified researchers on reasonable request.

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