OPEN

Haploidentical γδ T Cells Induce Complete Remission in Chemorefractory B-cell Non-Hodgkin Lymphoma

Anna Bold, Johannes Gaertner,* Alexander Bott,* Volker Mordstein,† Kerstin Schaefer-Eckart,* and Martin Wilhelm**

Summary: The transformation of chronic lymphocytic leukemia to an aggressive lymphoma, called Richter transformation, is often accompanied by resistance to chemotherapy and high mortality. Thus, novel therapeutic strategies are required for the successful treatment of these patients. One possibility is cellular immunotherapy with chimeric antigen receptor T cells. However, the time delay until cells are available and the limited number of effector cells due to the impaired immune system of these patients potentially compromises the efficacy of this approach. Another promising attempt might be the therapy with γδ T cells. Once activated, they exhibit various antitumor effects against several types of malignancies. Furthermore, they can be safely used in an allogeneic setting and can be multiplied in vivo as already demonstrated in clinical studies. In vitro data, in addition, show that the cytotoxicity of γδ T cells can be significantly enhanced by monoclonal antibodies. Here we present a patient, who suffered from Richter transformation and did not respond to several lines of immunochemotherapy. Due to the lack of further therapy options, we conducted an individual therapy with adoptive transfer of haploidentical γδ T cells combined with the application of the monoclonal antibody obinutuzumab. A histologically confirmed complete remission was achieved through this therapy approach, whereby relevant side effects were not seen. This case highlights the potential of γδ T cells and the feasibility of this therapeutic approach for further clinical trials.

Key Words: γδ T cells, obinutuzumab, lymphoma, allogeneic transplantation, Richter transformation

(*J Immunother* 2023;46:56–58)

CASE PRESENTATION

A 60-year-old man was diagnosed with chronic lymphatic leukemia (CLL) in 2008. Until April 2016, the patient had undergone 6 cycles of immunochemotherapy with rituximab and bendamustine. Since B-symptoms developed and the number of lymphocytes increased again in March 2020, a therapy with ibrutinib was started. In February 2021, a biopsy was taken because of the growth of cervical lymph nodes. Histologic infiltration of a diffuse large B cell lymphoma consistent with a Richter transformation was diagnosed. Computed tomographic (CT) imaging also revealed increased mediastinal, mesenterial and retroperitoneal lymph nodes, splenomegaly, and a thickened distal esophagus. Therefore, a gastroscopy was performed and adenocarcinoma of the esophagus was, in addition, diagnosed. Due to the aggressiveness of the disease, we decided to treat the lymphoma first. After 2 cycles of R-CHOP therapy, the cervical and mediastinal lymph nodes regressed in size while the retroperitoneal and mesenteric lymph nodes increased accompanied by ascites. Biopsy of a retroperitoneal lymph node revealed infiltration by the lymphoma and no progress of the esophagus carcinoma. In April 2021, the therapy was changed to gemcitabine and oxaliplatin, which could not control the disease. After treatment with rituximab, bendamustine, and polatuzumab vedotin, the lymphoma showed continuing progression and was considered as chemorefractory. Because of the lack of further therapy options, which would have been available in a timely manner, we decided to perform an experimental therapy with allogenic γδ T cells of his haploidentical daughter. This approach was approved by the Institutional Review Board and written informed consent was given by the patient and his daughter.

The rationale for this therapy was our in vivo experience with haploidentical γδ T cells and especially the in vitro finding that the activity of γδ T cells is strongly enhanced by the binding of an antibody to cell lines, in which γδ T cells alone did not show any cytotoxicity. Figure 1 shows the results of an exemplary in vitro cytotoxicity assay performed as described previously.[1] Mononuclear

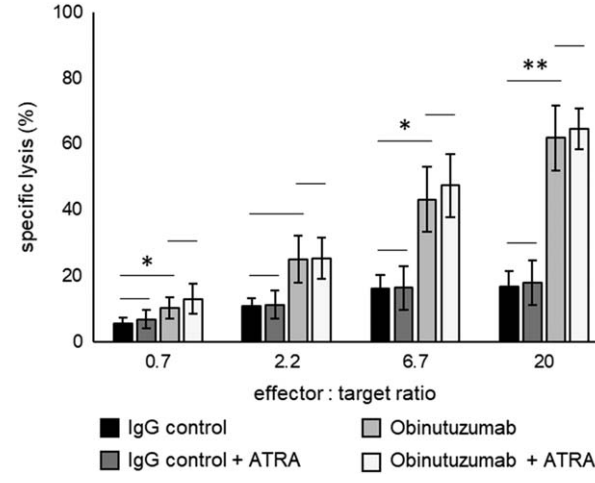

FIGURE 1. Enhancement of cytotoxicity of γδ T cells by obinutuzumab. Mononuclear cells isolated from healthy donors were cultured with zoledronate and interleukin-2. After 9 to 10 days, these effector cells consisting of over 90% γδ T cells were coincubated with Granta cells, which have been pretreated with 1 μM ATRA for 24 hours or not, and with 1 μg/mL obinutuzumab or 1 μg/mL of its unspecific isotype control. After coculture for 4 hours, lysed cells were analyzed by flow cytometry. Specific cell-mediated cytotoxicity is expressed as "specific lysis (%)," which is shown in different effector: target ratios. Data are presented as mean +/− standard error of the mean of 5 independent experiments. "*" indicates $P < 0.05$ and "**" indicates $P < 0.01$, calculated using the paired t test or the Wilcoxon test if parameters are not normally distributed. ATRA indicates all-trans retinoic acid.

Received for publication June 20, 2022; accepted December 7, 2022.

From the *Department of Hematology and Medical Oncology; and †Department of Pathology, Paracelsus Medical University, Klinikum Nuernberg, Nuremberg, Germany.

Reprints: Martin Wilhelm, Department of Hematology and Medical Oncology, Paracelsus Medical University, Klinikum Nuernberg, Prof-Ernst-Nathan-Street 1, Nuremberg 90419, Germany (e-mail: m_wilhelm@hotmail.de).

cells were isolated from 5 healthy donors and stimulated with zoledronate and interleukin-2 (Il-2) for 9 to 10 days resulting in a cell product consisting of 95.3+/−0.9% γδ T cells, 3.0+/−0.7% αβ T cells, and 0.8+/−0.2% natural killer cells. These effector cells were coincubated with Granta lymphoma cells pretreated with or without 1 μM all-trans retinoic acid (ATRA) for 24 hours and with 1 μg/mL obinutuzumab or its unspecific isotype control. After 4 hours, the lysed cells were measured by flow cytometry using 7-AAD. Specific cell-mediated cytotoxicity is expressed as "specific lysis (%)" and is calculated by the following formula: specific lysis (%) = (% 7-AAD+ target cells at the respective effector to target ratio − % 7-AAD+ target cells in target cell only culture)*100/(100 − % 7-AAD+ target cells in target cell only culture). As in this experimental setting, the effect of ATRA was minor and did not reach significance, the results show that the antibody obinutuzumab alone is able to significantly enhance the cytotoxicity of γδ T cells.

Figure 2 outlines the treatment protocol in June 2021. Nine days before the adoptive transfer of the haploidentical γδ T cells, the patient received 2×10^5 per kilogram body weight peripheral blood leukocytes of the daughter combined with high dose cyclophosphamide (50 mg/kg days −6 and −5) to postpone graft rejection according to the procedure in conventional allogeneic transplantation.[2] Similar to the procedure with chimeric antigen receptor (CAR) T cells, the patient received lymphodepleting therapy with 25 mg/m^2 fludarabine from day −6 until −2. The patient received neither further conditioning therapy nor immunosuppression.

In addition, a total dose of 1000 mg obinutuzumab was infused in 2 doses between days −3 and −2 to improve the cytotoxic activity of γδ T cells. Furthermore, 90 mg ATRA per day was given orally for 3 days before adoptive transfer to sensitize the tumor cells. Although ATRA did not show reproducible in vitro effects, reasons to include ATRA in the treatment protocol were evidence of increased cytotoxicity with other cell lines/antibodies and that human B cell lymphoma cell lines are highly sensitive to apoptosis induced by ATRA and interferon-γ[3] (own unpublished results).

For the donor innate lymphocyte infusion, a single unstimulated leukapheresis was performed from the daughter as described.[4] In brief, the cells were labeled with anti-αβ T cell receptor and CD19 antibodies after apheresis, both conjugated directly or indirectly through biotin to paramagnetic particles, and then processed with the fully automated device CliniMACS Plus (Miltenyi Biotec) according to the manufacturer's instructions to deplete the αβ T cells and B cells. The depletion was very effective leaving no significant number of αβ T cells (8.8×10^3/kg body weight) and B cells (1.1×10^3/kg body weight) in the transplant. The patient finally received 2.94×10^6 γδ T cells per kilogram body weight. Zoledronate was administered immediately after transfer and low dose Il-2 from day 1 until day 7 for stimulation of γδ T cells. On day 7, the administration of obinutuzumab was repeated to enhance the γδ T cell's cytotoxicity. Side effects like graft versus host reaction or cytokine release syndrome were not seen as expected from our previous study.[5] The patient received s.c. G-CSF and regeneration of leukocytes started on day 5. The T-cell chimerism analyzed on day 6 showed 2% of T cells, which proved the success of the allogeneic T cell transfer.

CT imaging 3 weeks after the γδ T cell infusion revealed a complete remission with only a few residual findings in retroperitoneal and mesenteric lymph nodes. Figure 3A shows an exemplary section of the CT scan before and after cellular therapy. In July 2021, the patient underwent surgery for his adenocarcinoma of the esophagus. No infiltrates of diffuse large B cell lymphoma and CLL were found in the 22 lymph nodes removed in this procedure, which confirmed the complete remission of the lymphoma (Fig. 3B).

In October 2021, 4 months after the experimental treatment, the patient presented to the clinic with a relapse of the aggressive lymphoma involving bone, liver, and lymph nodes. Flow cytometric analysis of the lymphoma cells showed antigen loss of CD20 and CD19. The patient died of the disease shortly thereafter.

DISCUSSION

Immunotherapy has become an important part of treatment for many malignant disorders. However, the therapeutic effects depend on the patient's immune system, which is often suppressed by the disease or previous therapies. Immunotherapies with CAR T cells or checkpoint inhibitors, partially overcome these restrictions. Nevertheless, only a small proportion of heavily pretreated patients benefits from these therapies in the long term.[6]

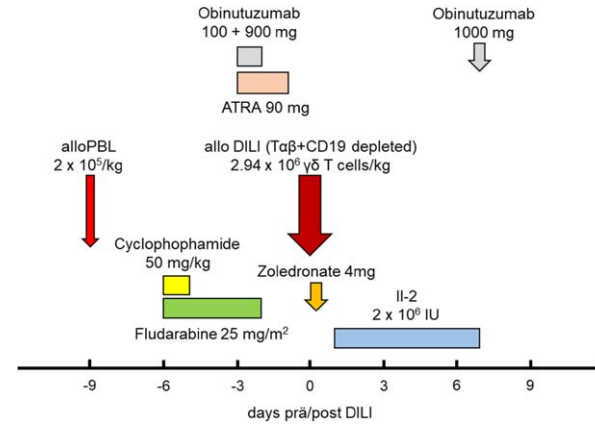

FIGURE 2. Treatment protocol. The timeline of the therapy is shown. The day of the adoptive transfer of the γδ T cells is designated as day 0. ATRA indicates all-trans retinoic acid; DILI, donor innate lymphocyte infusion; Il-2, Interleukin-2; IU, international units; PBL, peripheral blood leukocytes; Tαβ, αβ T cell receptor.

Another promising type of immunotherapy might be cellular therapy with γδ T cells, which represent about 5% of T cells in human peripheral blood and exhibit cytotoxic effects on malignant cells in vitro and in vivo.[1,7,8] γδ T cells differ from αβ T cells in their T cell receptor, whose antigen recognition is not restricted to major histocompatibility complexes. Therefore, no graft-versus-host-reaction is expected in a nonidentical transfer, as shown in our pilot study.[5] However, the response to therapy in these studies was very heterogeneous, which correlates with our in vitro data also showing varying antitumor activity of auto and allo-γδ T cells from patients or healthy donors. Recently, we have demonstrated that the cytotoxic potential of γδ T cells is significantly enhanced when combined with monoclonal antibodies.[1]

Because of the esophagus carcinoma, the patient was not able to receive CAR T cells and no other treatment options were available. In addition, as a result of multiple prior treatments, his own T cell activity was heavily suppressed. Therefore, we applied the γδ T cell therapy with the haploidentical cells of his daughter. In contrast to our previous clinical study, the patient was, in addition, treated with obinutuzumab to improve the cytotoxic effect of γδ T cells. The patient achieved a histologically proven complete remission with this protocol. Multiple chemotherapeutic drugs with or without monoclonal antibodies were not able to induce remission before, which is consistent with our and the other's experience with Richter transformation of CLL.[9] Therefore, we attribute this excellent result to our experimental protocol with γδ T cells. Although we have depleted the transplant of αβ T cells, we cannot exclude that other lymphocytes such as natural killer cells contributed to the antilymphoma response. However, stimulation of mononuclear cells with the combination of aminobisphosphonates and Il-2 is known to induce expansion and activation specifically of γδ T cells.[10]

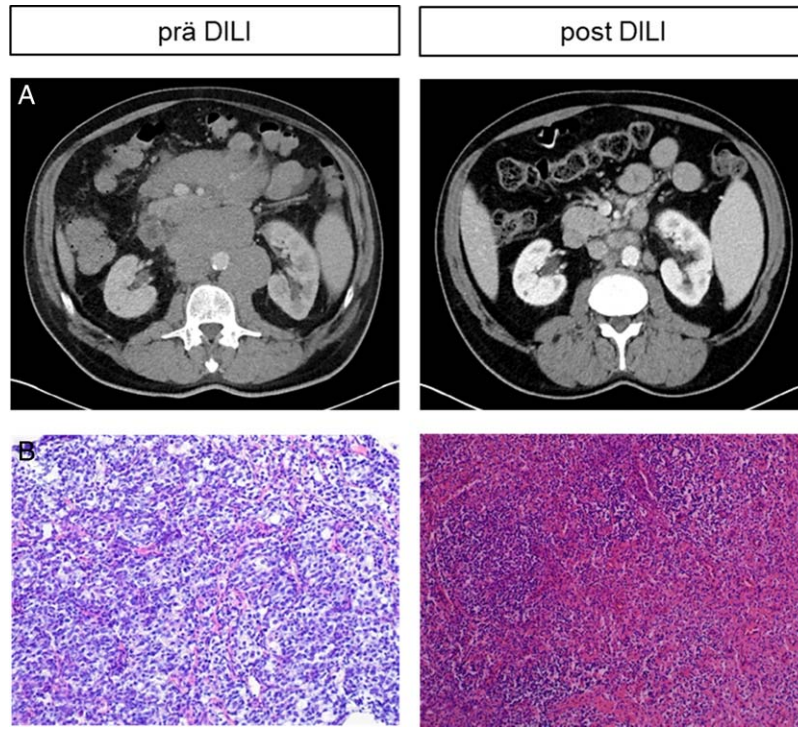

FIGURE 3. γδ T cell therapy induced complete remission. A, Abdominal computed tomography scans before and after the therapy with γδ T cells. The lymphomas significantly decreased in size after treatment. B, Microscopic images of lymph nodes examined before and after the therapy with γδ T cells. Left: diffuse infiltration of large lymphoid cells that have totally effaced the architecture. Right: normal architectural features with signs of lymphocyte depletion, fibrosis, hemorrhage, and no presence of diffuse large B cell lymphoma cells. DILI indicates donor innate lymphocyte infusion.

The patient relapsed a few months later with lymphoma cells, which have lost expression of CD20. In our opinion, this should not be interpreted as the ineffectiveness of the γδ T cell therapy. Even after a remission induced by CAR T cells, antigen-loss recurrences are not uncommon. The advantage of our approach is the possibility to combine antibodies against different targets potentially overcoming the problem of antigen escape.

CONCLUSION

Richter transformation is an aggressive disease and is usually unresponsive to immunochemotherapy as illustrated by this case. To our knowledge, the reported patient is the first case of a refractory lymphoma achieving complete remission after the treatment with a combination of haploidentical γδ T cells and a monoclonal antibody. This combination was based on our in vitro experiments and should be further investigated in clinical trials.

ACKNOWLEDGMENTS

The authors thank Heike Gross, Elisabeth Holzmann, Manuela Stuewe, and Hildegard Wessel for their expert technical assistance. Anna Bold was supported by "Verein Hilfe für Krebskranke e.V.".

Conflicts of Interest/Financial Disclosures

All authors have declared that there are no financial conflicts of interest with regard to this work.

REFERENCES

1. Hoeres T, Pretscher D, Holzmann E, et al. Improving immunotherapy against B-cell malignancies using γδ T-cell-specific stimulation and therapeutic monoclonal antibodies. *J Immunother*. 2019;42:331–344.
2. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14:641–650.
3. Niitsu N, Higashihara M, Honma Y. Human B-cell lymphoma cell lines are highly sensitive to apoptosis induced by all-trans retinoic acid and interferon-gamma. *Leuk Res*. 2002;26:745–755.
4. Smetak M, Kimmel B, Birkmann J, et al. Clinical-scale single-step CD4(+) and CD8(+) cell depletion for donor innate lymphocyte infusion (DILI). *Bone Marrow Transplant*. 2008;41:643–650.
5. Wilhelm M, Smetak M, Schaefer-Eckart K, et al. Successful adoptive transfer and in vivo expansion of haploidentical γδ T cells. *J Transl Med*. 2014;12:45.
6. Munshi NC, Anderson LD, Shah N, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. *N Engl J Med*. 2021;384:705–716.
7. Morita CT, Mariuzza RA, Brenner MB. Antigen recognition by human gamma delta T cells: pattern recognition by the adaptive immune system. *Springer Semin Immunopathol*. 2000;22:191–217.
8. Silva-Santos B, Serre K, Norell H. γδ T cells in cancer. *Nat Rev Immunol*. 2015;15:683–691.
9. Allan JN, Furman RR. Current trends in the management of Richter's syndrome. *Int J Hematol Oncol*. 2018;7:IJH09.
10. Wilhelm M, Kunzmann V, Eckstein S, et al. Gammadelta T cells for immune therapy of patients with lymphoid malignancies. *Blood*. 2003;102:200–206.

