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Letters to Blood

TO THE EDITOR:

Long-term outcomes of Sleeping Beauty–generated CD19-specific CAR T-cell therapy for relapsed-refractory B-cell lymphomas

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Patients with relapsed or refractory B-cell non-Hodgkin lymphoma have a poor prognosis. Autologous stem cell transplantation (ASCT) remains the only standard-of-care curative treatment of these patients. However, little progress has been made in improving transplant outcomes since the landmark study by the PARMA group in 1996.[1,2] Only 40% to 50% of patients can be cured with ASCT, and the majority of heavily pretreated (>2 lines of previous treatments) patients, or those with refractory disease at time of transplant, will eventually relapse.[3-6] Patients who relapse early after ASCT (within 12 months from transplantation) have a dismal outcome, with a median overall survival of 6.2 months.[5] Disease status at time of transplantation is among the strongest predictors for long-term survival after transplant; patients with complete remission (CR) at the time of the procedure have a median overall survival of >5 years.[3,5]

Anti-CD19 chimeric antigen receptor (CAR) T-cell therapy has emerged recently as one of the most successful practice-changing achievements over the past decade in the treatment of patients with relapsed or refractory B-cell lymphoid malignancies. Impressive response rates are noted in patients with multiply relapsed or refractory diffuse large B-cell lymphoma (DLBCL), including patients who relapsed after ASCT.[7,8] However, it is estimated that <30% to 35% might achieve long-term remissions with CAR T-cell therapy,[7,8] and longer follow-ups are eagerly awaited to assess for sustained durable remissions. Furthermore, patients who do not achieve CR, and those who relapse after CAR T-cell therapy, have very rapidly progressive disease and a poor prognosis.

Incorporation of CAR T-cell therapy into ASCT is hypothesized to improve response rates and decrease relapse after transplantation. We previously reported a first-in-human phase 1 feasibility clinical trial (#NCT00968760) with a novel approach in which T cells were genetically modified by using the Sleeping Beauty transposon/transposase system to express a second-generation CD19-specific CAR, cosignaling through CD3 and CD28.[9] The single-chain variable fragment was derived from the murine FMC63 monoclonal antibody. Patients with relapsed or refractory CD19$^+$ B-cell lymphomas were eligible. Nine patients were enrolled, but only 7 patients received treatment: 1 enrollee did not receive treatment because of manufacturing difficulty, and 1 patient could not receive a manufactured product because he was medically unstable.

Autologous CD19-specific CAR T cells were infused 2 days after autologous stem cell infusion conditioning with carmustine, etoposide, cytarabine, and melphalan. No unexpected acute toxicities were reported in the peri-transplant period.[9] Patients were subsequently enrolled in a long-term follow-up study (#NCT01492036), which included a 15-year follow-up during which patients will be tested annually for the persistence of genetically modified T cells using droplet digital polymerase chain reaction and flow cytometry. The study was institutional review board approved and was conducted in accordance with the Declaration of Helsinki. Here we report the long-term follow-up data after a median period of 4.7 years (range, 3.4-5.6 years). A total of 7 patients (median age, 52 years) have been treated and followed up between March 2013 and December 2018. Patient and disease characteristics are summarized in Table 1. Four patients had de novo or transformed DLBCL, and one each had relapsed primary central nervous system (CNS) lymphoma, low-grade follicular lymphoma, and mantle cell lymphoma. Genetic rearrangements and immunohistochemical stains MYC, BCL2, and BCL6 were not conducted for most patients because these tests were not universally available at the time of initiation of the study. The patients had a median of 3 previous lines of treatment (range, 2-4); 4 of these patients had >2 previous lines of therapy, and 3 of these 4 patients had residual disease at the time of transplantation. Patient number 3 received 2 previous lines of therapy, but he is high risk by virtue of his primary cutaneous DLBCL, leg type, at presentation, relapsed with CNS involvement before transplantation, and had double expressor lymphoma (co-expression of MYC and BCL2). Patient number 6 underwent transplantation for relapsed mantle cell lymphoma (late ASCT, which tends to do poorly compared with early transplantation). Two of the 3 patients with residual disease achieved CR after transplantation and CAR T-cell infusion, and both remain in CR. The third patient (primary CNS lymphoma) had initially stable disease but then experienced probable progression 6 months later with a small intracranial thalamic lesion that could not be biopsied. This patient was salvaged with a short course of dexamethasone and received lenalidomide and

Table 1. Patient characteristics and long-term outcomes

Patient*	Age, y	Sex	Histology	CNS involvement	Disease status at SCT†	Previous lines of therapy	T-cell dose level	Toxicity‡	Progressed	Status at last follow-up	Response duration at last follow-up (mo)	Persistence of CAR T-cells at last follow-up
1	59	M	Primary CNS DLBCL	Yes	Residual disease	4	$5 \times 10^5/m^2$	None	Yes	Alive, in remission	51.5	Not done
2	55	M	DLBCL	No	Residual disease	3	$5 \times 10^8/m^2$	None	No	Alive, in remission	66.5	Yes
3	36	M	DLBCL	Yes	CR	2	$5 \times 10^9/m^2$	None	No	Alive, in remission	42.5	No
4	48	M	Follicular	No	Residual disease	3	$5 \times 10^2/m^2$	None	No	Alive, in remission	60	Yes
5	61	M	Transformed DLBCL§	No	CR	2	$5 \times 10^8/m^2$	None	No	Alive, in remission	40.5	No
6	47	M	MCL	No	CR	2	$5 \times 10^9/m^2$	None	No	Died, in remission	29.5	Yes
7	58	M	Transformed DLBCLII	No	CR	3	$5 \times 10^8/m^2$	None	No	Alive, in remission	64	Yes

M, male; MCL, mantle cell lymphoma.

*At relapse: Patients 2, 3, and 7 have a low-intermediate, low, and high-intermediate Age-Adjusted International Prognostic Index (IPI), respectively. Patient 4 has intermediate 2 follicular lymphoma IPI. Patient 5 has low-intermediate IPI. Patient 6 has low MCL IPI.

†Patient 1 has residual disease according to magnetic resonance imaging of the brain. Patients 2 and 4 had residual disease according to positron emission tomography scan.

‡Unexpected toxicities not otherwise expected from ASCT.

§Transformed from low-grade follicular lymphoma.

‖Transformed from nodular Hodgkin lymphoma.

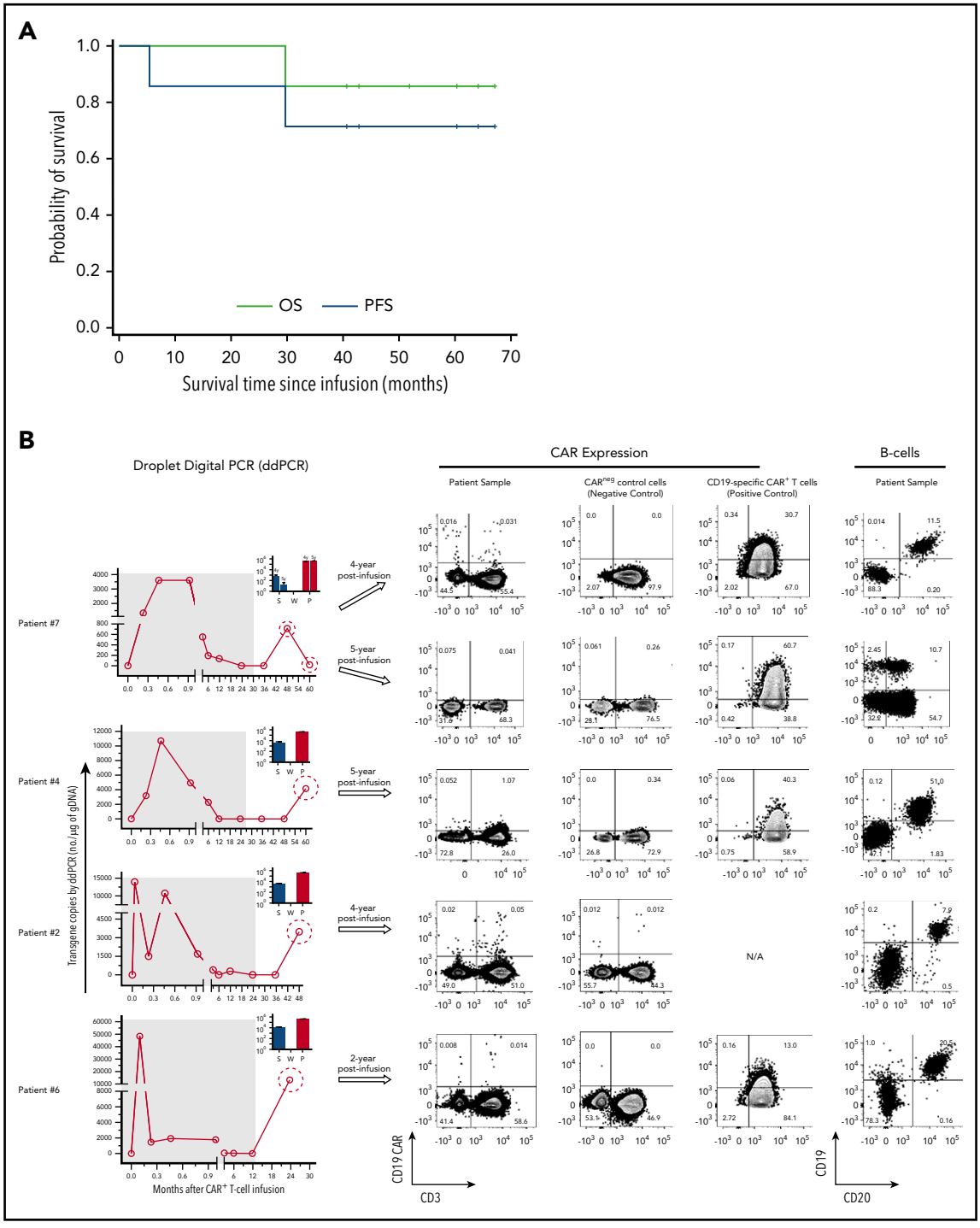

Figure 1. Patient outcomes and persistence of CAR T cells after therapy. (A) Progression-free survival (PFS) and overall survival (OS) of study patients. (B) Persistence of genetically modified T cells postinfusion was assessed by droplet digital PCR (ddPCR) and by flow cytometry as previously described.[9] ddPCR was used to evaluate integrated CAR transgene copy number per microgram of genomic DNA (gDNA). Insets (bar graphs) show CAR copy number (mean ± standard deviation [SD], log-axis) at the latest time point. CAR$^+$ Jurkat Clone 12 (stably expressing CD19-specific CAR [P]) was used as positive control, and Water (W) was used as negative control alongside patient sample (S). Using flow cytometry, total viable cells were gated to reveal CD3$^+$CAR$^+$ T cells and CD19$^+$CD20$^+$ B cells. Ex vivo expanded, genetically modified CD19-specific CAR^{pos} T cells were used as positive controls, and CAR^{neg} normal donor peripheral blood mononuclear cells or ex vivo expanded mock electroporated cells were used as negative controls.

rituximab, achieving CR within 2 months of salvage treatment and remains in remission at last follow up. None of the other enrolled patients had disease progression during the study period. Only 1 patient died (at 30 months from the transplant date) of a second malignancy (therapy-related myelodysplastic disorder) while in remission from his lymphoma. The 5-year progression-free survival and overall survival were 71% and 86%, respectively, for this study (Figure 1A).

Four of 6 patients have persistence of genetically modified T cells at the date of last follow-up (median time of persistence duration, 4.5 years; range, 2-5 years). To our knowledge, none of

the available CAR T-cell products being explored or used to treat lymphoma in the peri-transplant setting (average persistence cells <28 days)[10,11] or in the nontransplant setting (persistence noted up to 24 months) have reported persistence data lasting beyond 2 years.[7,8] As we previously reported,[9] B cells remained low for several months after CAR T-cell infusion but no long-term B-cell aplasia was observed. Of interest was the transient re-surgence of modified T cells in these patients with no gross evidence of progressive disease (Figure 1B). The resurgence of Sleeping Beauty–modified cells is believed to be related to the re-emergence of CD19$^+$ B cells.

Despite the small number of patients, this experience suggests that incorporation of anti-CD19 CAR T-cell therapy into ASCT is safe and could potentially improve cure rates after transplantation. This approach may particularly benefit patients deemed at very high risk of relapse after transplantation, such as those who received >2 lines of previous therapy and/or patients with residual disease at the time of the procedure. In our report, there were no unexpected notable toxicities from ASCT; specifically, there were no CAR T-cell–associated toxicities such as cytokine release syndrome or neurotoxicity. Other groups reported a similar toxicity profile but shorter follow-up of efficacy outcomes.[10] The decreased CAR T-cell–associated toxicities are likely related to decreased tumor burden at the time of transplantation and the intensity of the conditioning regimen. We speculate using CAR T cells in the adjuvant transplant setting, and the decreased CAR T-cell–associated toxicities will perhaps mitigate the costs associated with CAR T cells. This approach is potentially more cost-effective than using CAR T-cell therapy alone, if long-term remissions and decreased relapse after transplant are confirmed in phase 2 and 3 clinical trials.

Acknowledgment

This study was funded by Ziopharm Oncology, Inc.

Authorship

Contribution: P.K. and L.J.C. conceived and designed research; H.S., E.d.G., H.H., G.B., J.B., and P.K. assisted with correlative data; H.S. and H.H. assisted with the design of experiments; H.H. and E.J.S. assisted with procedures in the current Good Manufacturing Practice laboratory; J.M. and G.R. assisted with regulatory processes; S.A.S., L.J.C., and P.K. analyzed and interpreted data; S.A.S. wrote the first draft of the manuscript; S.A.S. and P.K. revised and contributed to writing the manuscript; M.Q., S.C., A.A., Y.N., K.R., D.M., U.P., C.H., E.J.S., R.E.C., and P.K. were involved in direct patient care and collaborated on the clinical trial; and S.A.S., M.Q, S.C., A.A., Y.N., K.R., D.M., U.P., C.H., E.J.S., W.G.W., H.K., R.E.C., L.J.C., and P.K. critically reviewed and edited the manuscript for important intellectual content.

Conflict-of-interest disclosure: E.d.G. is Executive Vice President and General Manager of Cell Therapy at Ziopharm Oncology, Inc.; and is a visiting scientist at MD Anderson Cancer Center. E.d.G. has financial relationships as follows: salary (Ziopharm Oncology) and ownership interest (Ziopharm Oncology). M.Q. has received research funding from Janssen, Bioline, and Angiocrine; and has been a consultant for Autolus Limited and Bioclinica Inc. G.B. and J.B. have financial relationships as follows: salary (Ziopharm Oncology) and ownership interest (Ziopharm Oncology). E.J.S. has served on advisory boards for Adaptimmune, CART Coop, Novartis, Axio, and Zelluna. L.J.C. is listed as inventors of certain intellectual property described in this article; is the CEO of Ziopharm Oncology, Inc.; and is a visiting scientist at MD Anderson Cancer Center. L.J.C. has financial relationships as follows: salary (Ziopharm Oncology, MD Anderson Cancer Center [until May 2015]), royalty (City of Hope, Immatics), contracted research (Ziopharm Oncology, Inc.), and ownership interest (Targazyme, Ziopharm Oncology, Immatics, Kiadis, and CellChorus). P.K. has served on advisory boards for Kite and Pfizer; received research support from Amgen and Ziopharm; and has been a consultant for Jazz. The remaining authors declare no competing financial interests.

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Footnote

All data-sharing requests should be sent to the corresponding author (e-mail: pkebriae@mdanderson.org).

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DOI 10.1182/blood.2019002920