STIMULUS REPORT

blood advances

Prevalence and variation of CHIP in patients with aggressive lymphomas undergoing CD19-directed CAR T-cell treatment

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Key Points

- CHIP is frequently observed in patients with r/r lymphoma undergoing CD19-directed CAR T-cell therapy.
- CHIP does not negatively influence the outcome of CD19-directed CAR T-cell therapy.

Inflammation plays an important role in chimeric antigen receptor (CAR) T-cell therapy, especially in the pathophysiology of cytokine-release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS). Clonal hematopoiesis of indetermined potential (CHIP) has also been associated with chronic inflammation. The relevance of CHIP in the context of CAR T-cell treatment is widely unknown. We evaluated the prevalence of CHIP, using a targeted deep sequencing approach, in a cohort of patients with relapsed/refractory (r/r) B-cell non-Hodgkin lymphoma before and after CAR T-cell treatment. The aim was to define the prevalence and variation of CHIP over time and to assess the influence on clinical inflammation syndromes (CRS/ICANS), cytopenia, and outcome. Overall, 32 patients were included. CHIP was found in 11 of 32 patients (34%) before CAR T-cell therapy. CHIP progression was commonly detected in the later course. Patients with CHIP showed a comparable response rate to CAR T-cell treatment but had an improved overall survival (not reached vs 265 days, $P = .003$). No significant difference was observed in terms of the occurrence and severity of CRS/ICANS, therapeutic use of tocilizumab and glucocorticosteroids, paraclinical markers of inflammation (with the exception of ferritin), or dynamics of hematopoietic recovery. CHIP is commonly observed in patients undergoing CD19-directed CAR T-cell therapy and is not associated with an inferior outcome.

Introduction

Chimeric antigen receptor (CAR)-modified T cells targeting CD19 are approved for the treatment of patients with certain relapsed/refractory (r/r) aggressive B-cell lymphomas. In addition to relapse, the commonly observed adverse events include cytokine release syndrome (CRS), immune effector cell–associated neurotoxicity syndrome (ICANS), and protracted cytopenia. CRS represents a prototypic inflammatory state that is induced by hyperactivation of diverse immune cells, including the myeloid lineage.[1] The pathophysiology of ICANS and prolonged cytopenia remains a matter of debate; however, inflammation has also been suggested to play a decisive role.[2-4]

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The full-text version of this article contains a data supplement.

Clonal hematopoiesis evolves from a somatically mutated hematopoietic stem cell and its progeny. Clonal hematopoiesis of indeterminate potential (CHIP) is defined as the presence of cancer-associated driver mutations with a variant allele frequency (VAF) $\geq$ 2% in subjects without hematologic abnormalities.[5] The prevalence of CHIP in individuals >65 years of age is >10%. CHIP has been associated with an increased risk for transformation to acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS), as well as with the occurrence of chronic inflammatory disorders, especially cardiovascular diseases.[6] Preclinical data suggest that inflammation is driven by CHIP-affected monocytic cells[7,8] and that inflammation even promotes clonal expansion, leading to a vicious circle.[9,10]

The relevance of CHIP in the context of CAR T-cell treatment is widely unknown.[11,12] Therefore, we longitudinally evaluated the prevalence of CHIP in patients with r/r B-cell non-Hodgkin lymphoma undergoing CAR T-cell treatment and assessed the influence on clinical inflammation syndromes (CRS/ICANS), cytopenia after CAR T-cell therapy, and outcome.

Methods

This study included 32 patients who were consecutively treated with CD19-directed CAR T-cells for r/r B-cell non-Hodgkin lymphoma between October of 2019 and August of 2021 (institutional approval number: BO-EK-266062020). All patients gave written informed consent. The prevalence of CHIP was analyzed before lymphodepleting chemotherapy to assess the impact of CHIP on CRS, ICANS, cytopenia, and outcome after CAR T-cell infusion. A possible mutual interference was evaluated by sequential CHIP assessment after CAR T-cell treatment. Therefore, an established targeted deep sequencing approach was used as previously described and outlined in supplemental Methods.[13] For better understanding, the term "CHIP" is uniformly used throughout the article, formally neglecting peripheral blood count criteria. The statistical methods used are outlined in detail in supplemental Methods.

Results and discussion

The median patient age was 62 years (range, 37-82 years). Most patients were extensively pretreated at the time of CAR T-cell infusion (median 4 lines of prior therapy). Only 40% of the patients achieved an objective response (partial or complete remission) to the last therapy before CAR T-cell infusion (Table 1).

Of the patients, 84% developed CRS (any grade), including 46% with grade $\geq$ 2 CRS. In addition, grade $\geq$ 2 ICANS was observed in 27% of patients (Table 1).

Most patients developed profound and prolonged cytopenia after CAR T-cell treatment. The prevalence of severe thrombocytopenia (platelets $< 50 \times 10^9$ per liter) was 70% and 47% at days +28 and +56, respectively, and severe neutropenia (neutrophils $< 1 \times 10^9$ per liter) was seen in 63% and 39% of the patients at days +28 and +56, respectively.

Somatic mutations in peripheral blood, indicative of CHIP, were found in 11 of 32 patients (34%) before CAR T-cell therapy (Figure 1). The prevalence of CHIP was not influenced by the lines of prior therapy. The exact mutational pattern is displayed in supplemental Table 1.

The patient cohort was divided according to the presence of CHIP (CHIP group, n = 11) or its absence (non-CHIP group, n = 21) before lymphodepletion (Table 1). Patients in the CHIP group were older (69 vs 58 years, $P = .014$). No significant difference was observed between the 2 groups in terms of the occurrence and severity of CRS and ICANS or the therapeutic use of tocilizumab and glucocorticosteroids. Paraclinical markers of inflammation did not differ, with the exception of ferritin, which was higher in non-CHIP patients. Furthermore, the dynamics of hematopoietic recovery was indistinguishable between patients in the CHIP and non-CHIP groups (Table 1; supplemental Figure 2).The overall response rate after CAR T-cell therapy did not depend on the CHIP status (CHIP vs non-CHIP: 90% vs 80%; $P = .3$; Table 1) and was not influenced by the VAF of the CHIP lesions (supplemental Figure 3). After a median follow-up of 213 days (range, 9-714 days), 64% of the patients in the CHIP group had an ongoing response compared with 35% of the patients in the non-CHIP group (median event-free survival: not reached vs 77 days; $P = .061$; supplemental Figure 1A). This converts into a median overall survival of not reached vs 265 days ($P = .003$; supplemental Figure 1B).

CHIP progression, defined by the occurrence of new mutations or an increase in the VAF of preexisting clones, was commonly detected in the course of follow-up. Notably, CHIP progression was not associated with the occurrence of severe CRS (grade $\geq$ 2).

Thus far, the potential impact of CHIP on adverse events and clinical outcomes of CAR T-cell therapy has not been studied in depth.[12] The prevalence of CHIP is generally known to be increased in older individuals and patients with prior exposure to cytotoxic agents.[14] Therefore, patients with r/r disease are likely to have an increased prevalence of CHIP at the time of CAR T-cell treatment. This was confirmed in the current cohort; CHIP was detected in 34% of the patients before CAR T-cell therapy.

The presence of CHIP has been associated with various inflammatory conditions (eg, cardiovascular disease or autoinflammatory syndromes). Preclinical studies in *TET2*-mutant mice have suggested an interleukin-6–dependent inflammation pathway,[9] a cytokine that is also known to be involved in the inflammatory adverse events associated with CAR T-cell therapy. Thus, the presence of CHIP may potentiate adverse events of CAR T-cell therapy, especially CRS or prolonged cytopenia. However, we did not observe any association between the presence of CHIP and increased clinical inflammation or delayed hematopoietic recovery in the current cohort, which could be explained, in part, by the small sample size. Furthermore, the clinical definition of CRS represents the inflammation storm immediately after CAR T-cell infusion, which is usually limited to a few days. In contrast, CHIP may maintain a subclinical CRS with a long-lasting and ongoing chronic inflammatory micromilieu similar to what is termed "inflammaging" in older people.[15]

Interestingly, trends toward a better event-free survival and a prolonged overall survival after CAR T-cell therapy were found in patients in the CHIP group. In this context, few genes that are frequently found to be mutated in CHIP are the main regulators of lymphocyte activity and, therefore, may influence the effectiveness of CAR T cells, which has been reported in murine models and a clinical case study.[16,17] Furthermore, clonal expansion was observed in most patients with ongoing responses in the follow-up after CAR T-cell therapy. Therefore, an important question will be whether CHIP progression related to CAR T-cell therapy further enhances

Table 1. Patient characteristics, CAR T-cell treatment, and response of the entire cohort and subgroups separated by the presence of CHIP before CAR T-cell therapy

Variable	All patients (N = 32)	CHIP group (n = 11)	Non-CHIP group (n = 21)	*P*
Sex				1.00
Male	19/32 (59)	7/11 (64)	12/21 (57)	
Female	13/32 (41)	4/11 (36)	9/21 (43)	
Age, median (range), y	62 (37-82)	69 (56-82)	58 (37-77)	.01
IPI, median	2	2	3	.20
Prior lines of therapy, median (range), n	4 (2-6)	3 (2-6)	4 (2-6)	.36
Remission before CAR T-cell treatment				.11
CR	2/32 (6)	2/11 (18)	0/21 (0)	
PR	11/32 (34)	5/11 (46)	6/21 (28)	
SD	6/32 (19)	1/11 (9)	5/21 (24)	
PD	13/32 (41)	3/11 (27)	10/21 (48)	
CAR T-cell product				.35
Axicabtagene ciloleucel	20/32 (62)	5/11 (45)	15/21 (71)	
Tisagenlecleucel	8/32 (25)	4/11 (36)	4/21 (19)	
Other	4/32 (13)	2/11 (19)	2/21 (10)	
CRS maximum*				.37
0	5/32 (16)	3/11 (27)	2/21 (10)	
1	12/32 (38)	5/11 (45)	7/21 (33)	
2	11/32 (34)	2/11 (19)	9/21 (43)	
3	4/32 (12)	1/11 (9)	3/21 (14)	
4	0/32 (0)	0/11 (0)	0/21 (0)	
ICANS, maximum*				.58
0	17/32 (53)	8/11 (73)	9/21 (43)	
1	6/32 (19)	1/11 (9)	5/21 (24)	
2	4/32 (12)	1/11 (9)	3/21 (14)	
3	1/32 (4)	0/11 (0)	1/21 (5)	
4	4/32 (12)	1/11 (9)	3/21 (14)	
Use of tocilizumab	22/32 (69)	6/11 (55)	16/21 (76)	.39
Use of steroids	15/32 (47)	3/11 (27)	12/21 (57)	.22
Transfusion support after day +28				
Red blood cells	18/30 (60)	3/10 (30)	15/20 (75)	.05
Platelets	19/29 (66)	3/9 (33)	16/20 (80)	.04
Best response to CAR T-cell treatment†				.30
CR	9/31 (29)	5/11 (45)	4/20 (20)	
PR	17/31 (55)	5/11 (45)	12/20 (60)	
SD	3/31 (10)	0/11 (0)	3/20 (15)	
PD	2/31 (6)	1/11 (9)	1/20 (5)	
Response at last follow-up†				.36
CR	5/31 (16)	3/11 (27)	2/20 (10)	
PR	9/31 (29)	4/11 (36)	5/20 (25)	
SD	2/31 (6)	0/11 (0)	2/20 (10)	
PD	15/31 (48)	4/11 (36)	11/20 (55)	
Death after CAR T-cell treatment	11/32 (34)	0/11 (0)	11/21 (5)	.01

Unless otherwise noted, data are n/N (%).
CR, complete response; IPI, International Prognostic Index; PD, progressive disease; PR, partial remission; SD, stable disease.
*Assessment according to the American Society for Transplantation and Cellular Therapy grading system.[19]
†Response was evaluated according to Lugano criteria.

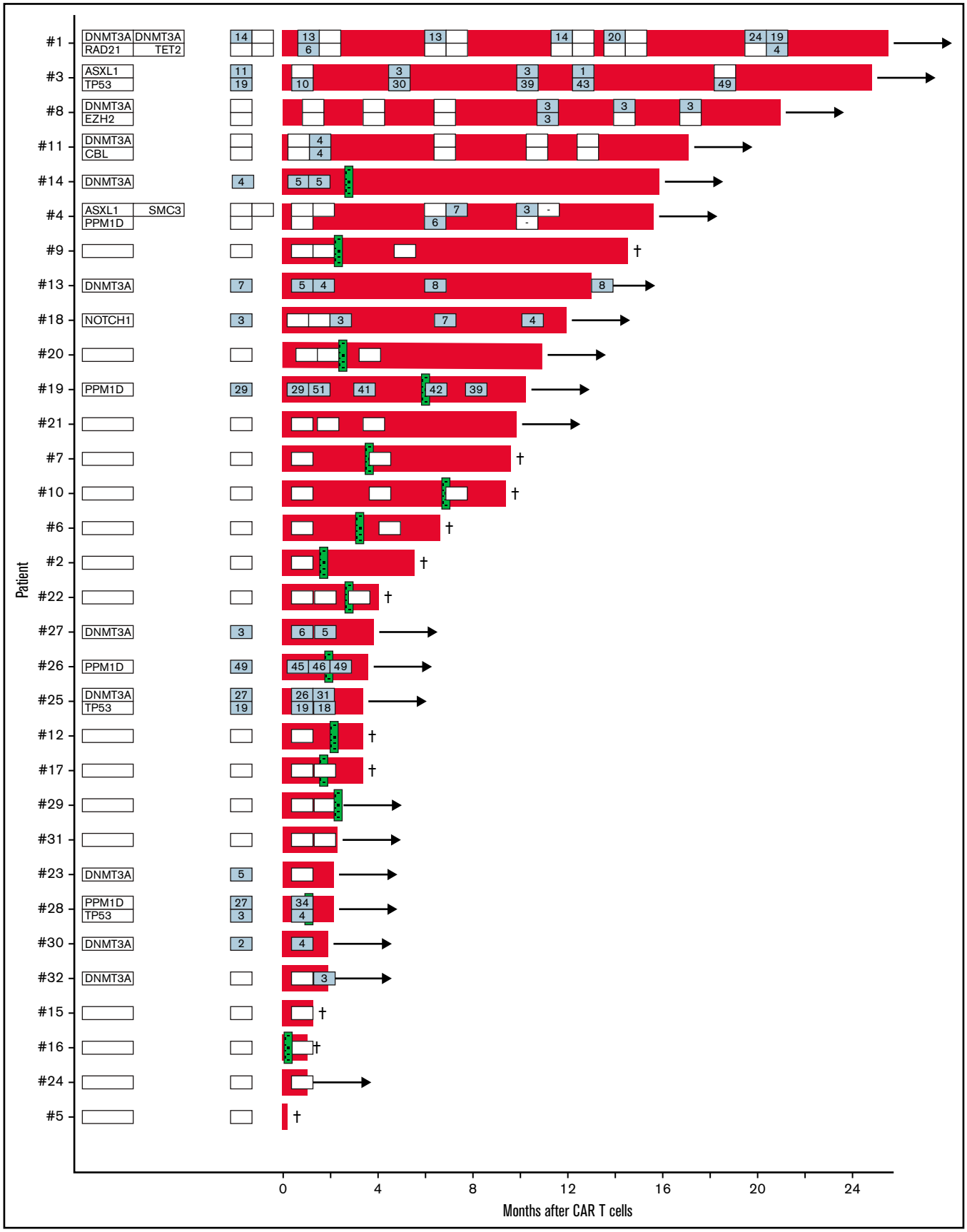
Patient
#1
DNMT3A RAD21
DNMT3A TET2
14
13 6
13
14
20
24 19 4
#3
ASXL1 TP53
11 19
10
3 30
3 39
1 43
49
#8
DNMT3A EZH2
3 3
3
3
#11
DNMT3A CBL
4 4
#14
DNMT3A
4
5 5
#4
ASXL1 PPM1D
SMC3
7 6
3 -
-
#9
†
#13
DNMT3A
7
5 4
8
8
#18
NOTCH1
3
3
7
4
#20
#19
PPM1D
29
29 51
41
42
39
#21
#7
†
#10
†
#6
†
#2
†
#22
†
#27
DNMT3A
3
6 5
#26
PPM1D
49
45 46 49
#25
DNMT3A TP53
27 19
26 31 19 18
#12
†
#17
†
#29
#31
#23
DNMT3A
5
#28
PPM1D TP53
27 3
34 4
#30
DNMT3A
2
4
#32
DNMT3A
3
#15
†
#16
†
#24
#5
†
0
4
8
12
16
20
24
Months after CAR T cells

Figure 1.

the risk of developing therapy-related myeloid neoplasms.[18] Thus far, in our cohort 1 patient developed a clinically relevant myelodysplastic syndrome. This patient received an allogeneic stem cell transplantation in the later course.

Our data have to be interpreted with caution and can only serve as a hypothesis-generating study, especially because of the small number of patients included. Other variables with a known influence on outcome in this patient cohort might also affect survival and influence the results of this study, reflecting the natural limitations of a univariate analysis. Nevertheless, other cellular therapies in patients with lymphoma were reported to be influenced negatively by CHIP, which we did not observe.[11] In the future, larger studies with longitudinal single-cell analysis of various immune cells, including the CAR T cells themselves, are necessary to perform reliable multivariate analyses and to elucidate the influence of CHIP-associated somatic mutations on the outcome of CAR T-cell treatment.

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Authorship

Contribution: R.T., F.K., C.T., and M.v.B. designed the study and analyzed data; M.K. performed statistical analyses; S.S., L.R., S.H., and C.T. performed next-generation sequencing and analyzed the experimental data; R.T., T. Kretschmann, K.E.-H., T. Krüger, K.S., J.M.M., K.T.-G., and M.v.B. followed up on clinical data; R.T. and M.v.B. interpreted the clinical data; and R.T., F.K., M.B., C.T., and M.v.B. wrote the manuscript; and all authors read and approved the final version of the manuscript.

Conflict-of-interest disclosure: C.T. is a co-owner and Chief Executive Officer of AgenDix GmbH. The remaining authors declare no competing financial interests

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References

1. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat Med.* 2018;24(6):731-738.
2. Jain T, Knezevic A, Pennisi M, et al. Hematopoietic recovery in patients receiving chimeric antigen receptor T-cell therapy for hematologic malignancies. *Blood Adv.* 2020;4(15):3776-3787.
3. Norelli M, Camisa B, Barbiera G, et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat Med.* 2018;24(6):739-748.
4. Rejeski K, Perez A, Sesques P, et al. CAR-HEMATOTOX: a model for CAR T-cell-related hematologic toxicity in relapsed/refractory large B-cell lymphoma. *Blood.* 2021;138(24):2499-2513.
5. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood.* 2015;126(1):9-16.
6. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med.* 2014;371(26):2488-2498.
7. Cull AH, Snetsinger B, Buckstein R, Wells RA, Rauh MJ. Tet2 restrains inflammatory gene expression in macrophages. *Exp Hematol.* 2017;55:56-70.e13.
8. Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science. 2017;355(6327):842-847.
9. Cai Z, Kotzin JJ, Ramdas B, et al. Inhibition of inflammatory signaling in Tet2 mutant preleukemic cells mitigates stress-induced abnormalities and clonal hematopoiesis. *Cell Stem Cell.* 2018;23(6):833-849.e5.
10. Abegunde SO, Buckstein R, Wells RA, Rauh MJ. An inflammatory environment containing TNFα favors Tet2-mutant clonal hematopoiesis. *Exp Hematol.* 2018;59:60-65.
11. von Bonin M, Jambor HK, Teipel R, et al. Clonal hematopoiesis and its emerging effects on cellular therapies. *Leukemia.* 2021;35(10):2752-2758.
12. Miller PG, Sperling AS, Brea EJ, et al. Clonal hematopoiesis in patients receiving chimeric antigen receptor T-cell therapy. *Blood Adv.* 2021;5(15):2982-2986.
13. Stasik S, Schuster C, Ortlepp C, et al. An optimized targeted next-generation sequencing approach for sensitive detection of single nucleotide variants. *Biomol Detect Quantif.* 2018;15:6-12.

Figure 1. CHIP and mutational burden over time. Assessment of CHIP at various time points before and after CAR T-cell infusion. All mutated genes detected over time are displayed for each patient. White boxes indicate that no CHIP-associated mutation is detectable. Gray boxes indicate a CHIP-associated mutation, and the number represents the VAF. Suspected germline variants are not depicted. White boxes with a black dot represent a different next-generation sequencing panel for which not all respective targets were included. Overall survival is shown for each patient (red bar). Progression/relapse is represented by a dotted green box. Patients alive at the last follow-up are marked with black arrows. Mortality (any cause) is indicated by a black cross.

14. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal hematopoiesis associated with adverse outcomes after autologous stem-cell transplantation for lymphoma. *J Clin Oncol.* 2017;35(14):1598-1605.

15. Kovtonyuk LV, Fritsch K, Feng X, Manz MG, Takizawa H. Inflamm-aging of hematopoiesis, hematopoietic stem cells, and the bone marrow microenvironment. *Front Immunol.* 2016;7(NOV):502.

16. Carty SA, Gohil M, Banks LB, et al. The loss of TET2 promotes $CD8^+$ T cell memory differentiation. *J Immunol.* 2018;200(1):82-91.

17. Fraietta JA, Nobles CL, Sammons MA, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. *Nature.* 2018; 558(7709):307-312.

18. Gillis NK, Ball M, Zhang Q, et al. Clonal haemopoiesis and therapy-related myeloid malignancies in elderly patients: a proof-of-concept, case-control study. *Lancet Oncol.* 2017;18(1):112-121.

19. Lee DW, Santomasso BD, Locke FL, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant.* 2019;25(4):625-638.