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

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Abstract:
Inflammation plays an important role in 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 currently widely unknown. We longitudinally evaluated the prevalence of CHIP, using a targeted deep sequencing approach in a cohort of patients with r/r B-NHL 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 OS (not reached vs. 265 days, p=0.003). No significant difference was observed in terms of the occurrence and severity of CRS/ICANS, therapeutic usage of tocilizumab and glucocorticosteroids, paraclinical markers of inflammation (except 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.

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Short title:
CHIP in CAR–T-cell treatment

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Abstract

Inflammation plays an important role in 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 currently widely unknown. We longitudinally evaluated the prevalence of CHIP, using a targeted deep sequencing approach in a cohort of patients with r/r B-NHL 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 OS (not reached vs. 265 days, p=0.003). No significant difference was observed in terms of the occurrence and severity of CRS/ICANS, therapeutic usage of tocilizumab and glucocorticosteroids, paraclinical markers of inflammation (except 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.

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.
Introduction

Chimeric antigen receptor (CAR)-modified T-cells targeting CD19 are approved for the treatment of patients with certain relapsed and refractory 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 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.\(^2-4\)

Clonal hematopoiesis (CH) 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) of at least 2% in subjects without hematologic abnormalities.\(^5\) The prevalence of CHIP in individuals >65 years of age is >10%. CHIP has been associated not only with an increased risk of transformation to AML/MDS but also 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 currently widely unknown.\(^11,12\) We therefore longitudinally evaluated the prevalence of CHIP in patients with relapsed/refractory B-cell non-Hodgkin lymphoma (r/r B-NHL) 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 consecutively treated with CD19-directed CAR-T-cells for r/r B-NHL between October 2019 and August 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 the supplement. For better understanding the term CHIP is uniformly used throughout the manuscript, formally neglecting peripheral blood count criteria. The respective statistical methods are outlined in detail in the supplement.

**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, four 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 CRS ≥ grade 2. In addition, ICANS ≥ grade 2 was observed in 27% of the patients (Table 1).

Most patients developed profound and prolonged cytopenia after CAR-T-cell treatment. The prevalence of severe thrombocytopenia (<50 Gpt/L) was 70% and 47% at days +28 and +56, respectively. Severe neutropenia (<1 Gpt/L) 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 Supplementary Table 1.

The patient cohort was divided according to the presence (CHIP group, n = 11) and absence of CHIP (non-CHIP group, n = 21) before lymphodepletion (Table 1). Patients in the CHIP group were older (69 vs. 58 years, p = 0.014). No significant difference was observed between the two groups in terms of the occurrence and severity of CRS and ICANS or therapeutic usage of tocilizumab and glucocorticosteroids. Paraclinical markers of
inflammation did not differ, except ferritin, which was higher in non-CHIP patients. Furthermore, dynamics of hematopoietic recovery was indistinguishable between patients of the CHIP and non-CHIP groups (Table 1; Supplementary Figure 2A–C). The overall response rate (ORR) after CAR-T-cell therapy did not depend on the CHIP status (ORR CHIP vs. non-CHIP: 90% vs. 80%, p = 0.3; Table 1) and was not influenced by the VAF of the CHIP lesions (Supplementary 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 EFS: not reached vs. 77 days; p = 0.061; Supplementary Figure 1A). This converts into a median overall survival of not reached vs. 265 days (p = 0.003; Supplementary Figure 1B).

CHIP progression, defined either 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 occurrence of severe CRS (≥ grade 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. The prevalence of CHIP is generally known to be increased in older individuals and patients with prior exposure to cytotoxic agents. Therefore, patients with relapsed and refractory 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 wherein CHIP was detected in 34% of the patients before CAR-T-cell therapy.

The presence of CHIP has been associated with various inflammatory conditions, e.g., cardiovascular disease or autoinflammatory syndromes. Preclinical studies in TET2-mutant mice have suggested an IL-6-dependent inflammation pathway, a cytokine also known to be involved in the inflammatory adverse events of 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 partly explained 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 *inflamming*. In older people.\(^{15}\)

Interestingly, a trend toward a better EFS and a prolonged OS after CAR-T-cell therapy was found in the patients of the CHIP group. In this context, few genes 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, if CHIP progression related to CAR-T-cell therapy further enhances the risk of development of therapy related myeloid neoplasms.\(^{18}\) Thus far, in our cohort one patient developed a clinically relevant myelodysplastic syndrome. This patient received an allogeneic stem cell transplantation in the later course.

Nevertheless, our data have to be interpreted with caution and can only serve as a hypothesis-generating impulse especially regarding the small number of patients of this preliminary study. Other variables with known influence on outcome in this patient cohort might also affect survival and possibly influence the results of this study, reflecting the natural limitations of a univariate analysis. Nevertheless, other cellular therapies in lymphoma patients have reported a negative influence of 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: RT, FK, CT and MvB designed the study and analyzed the data; MK performed the statistical analysis; SS, LR, SH and CT performed the NGS and analyzed the experimental data; RT, TK, KHE, TKR, KS, JMM, KT and MvB followed up on clinical data; RT and MvB interpreted the clinical data; RT, FK, MB, CT and MvB wrote the manuscript; all authors read and approved the final manuscript.

Conflict-of-interest disclosure: CT declares co-ownership and CEO position of AgenDix GmbH, Dresden, Germany. All other authors declare no competing interests.

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### Table 1: Patient characteristics, CAR-T-cell treatment and response of the entire cohort and divided 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, n/N (%)**                              |                     |                   |                        |     |
| • Male                                        | 19/32 (59)          | 7/11 (64)         | 12/21 (57)             | p = 1.00 |
| • Female                                      | 13/32 (41)          | 4/11 (36)         | 9/21 (43)              |     |
| **Age, median (range)**                       | 62 (37–82)          | 69 (56–82)        | 58 (37–77)             | p = 0.01 |
| **IPI, median**                               | 2                   | 2                 | 3                      | p = 0.20 |
| **Prior lines of therapy, median (range)**    | 4 (2–6)             | 3 (2–6)           | 4 (2–6)                | p = 0.36 |
| **Remission before CAR-T-cell treatment, n/N (%)** |                     |                   |                        |     |
| • CR                                          | 2/32 (6)            | 2/11 (18)         | 0/21 (0)               | p = 0.11 |
| • PR                                          | 11/32 (34)          | 5/11 (45)         | 6/21 (29)              |     |
| • SD                                          | 6/32 (19)           | 1/11 (9)          | 5/21 (24)              |     |
| • PD                                          | 13/32 (41)          | 3/11 (27)         | 10/21 (48)             |     |
| **CAR-T product, n/N (%)**                    |                     |                   |                        | p = 0.35 |
| • Axicabtagene cilooleucel                    | 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 † max, n/N (%)**                        |                     |                   |                        | p = 0.37 |
| • 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 † max, n/N (%)**                      |                     |                   |                        | p = 0.58 |
| • 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)              |     |
| **Tocilizumab used, n/N (%)**                 | 22/32 (69)          | 6/11 (55)         | 16/21 (76)             | p = 0.39 |
| **Steroids used, n/N (%)**                    | 15/32 (47)          | 3/11 (27)         | 12/21 (57)             | p = 0.22 |
### Transfusion support >day +28, n/N (%)

- **Red blood cells**: 18/30 (60) 3/10 (30) 15/20 (75) \( p = 0.05 \)
- **Platelets**: 19/29 (66) 3/9 (33) 16/20 (80) \( p = 0.04 \)

### Best response* to CAR-T, n/N (%)

- **CR**: 9/31 (29) 5/11 (45) 4/20 (20) \( p = 0.30 \)
- **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 FU, n/N (%)

- **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) \( p = 0.36 \)

### Death after CAR-T-cell treatment, n/N (%)

|           | 11/32 (34) | 0/11 (0) | 11/21 (52) | \( p = 0.01 \) |
|-----------|------------|----------|------------|---------------|

*Response was evaluated according to the Lugano criteria.
†Assessment according to the ASCTC-grading scale. 19
Figure legends

Figure 1. CHIP and mutational burden over time. Assessment of CHIP at various points before and after CAR-T-cell infusion. All mutated genes detected over time are displayed for each patient. *White blank boxes* indicate that no CHIP-associated mutation is detectable at the respected time. *Dark gray boxes* indicate a CHIP-associated mutation, and the *number* represents the respective VAF for a specific time. Suspected germline variants are not depicted. *White boxes with a black dot* mark a different NGS-panel, in which not all respective targets were included. Overall survival is reported for each patient (*black bar*). The occurrence of progression/relapse is marked with a *dotted box*. Patients alive at the last follow-up without relapse/progression are marked with *black arrows*. Mortality (any cause) is indicated with a *black cross.*
