

BMJ Open Treatment of patients with relapsed or refractory CD19+ lymphoid disease with T lymphocytes transduced by RV-SFG. CD19.CD28.4-1BBzeta retroviral vector: a unicentre phase I/II clinical trial protocol

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ABSTRACT

Introduction Chimeric antigen receptor (CAR) T cells spark hope for patients with CD19+ B cell neoplasia, including relapsed or refractory (r/r) acute lymphoblastic leukaemia (ALL) or r/r non-Hodgkin's lymphoma (NHL). Published studies have mostly used second-generation CARs with 4-1BB or CD28 as costimulatory domains. Preclinical results of third-generation CARs incorporating both elements have shown superiority concerning longevity and proliferation. The University Hospital of Heidelberg is the first institution to run an investigator-initiated trial (IIT) CAR T cell trial (Heidelberg Chimeric Antigen Receptor T cell Trial number 1 [HD-CAR-1]) in Germany with third-generation CD19-directed CAR T cells.

Methods and analysis Adult patients with r/r ALL (stratum I), r/r NHL including chronic lymphocytic leukaemia, diffuse large B-cell lymphoma, follicular lymphoma or mantle cell lymphoma (stratum II) as well as paediatric patients with r/r ALL (stratum III) will be treated with autologous T-lymphocytes transduced by third-generation RV-SFG.CD19.CD28.4-1BB zeta retroviral vector (CD19.CAR T cells). The main purpose of this study is to evaluate safety and feasibility of escalating CD19.CAR T cell doses ($1-20 \times 10^6$ transduced cells/ m^2) after lymphodepletion with fludarabine (flu) and cyclophosphamide (cyc). Patients will be monitored for cytokine release syndrome (CRS), neurotoxicity, i.e. CAR-T-cell-related encephalopathy syndrome (CRES) and/or other toxicities (primary objectives). Secondary objectives include evaluation of *in vivo* function and survival of CD19.CAR T cells and assessment of CD19.CAR T cell antitumour efficacy. HD-CAR-1 as a prospective, monocentric trial aims to make CAR T cell therapy accessible to patients in Europe. Currently, HD-CAR-1 is the first and only CAR T cell IIT in Germany. A third-generation Good Manufacturing Practice (GMP) grade retroviral vector, a broad spectrum of NHL, treatment of paediatric and adult ALL patients and inclusion of patients even after allogeneic stem cell transplantation (alloSCT) make this trial unique.

Strengths and limitations of this study

- First investigator-initiated trial with CAR T cells in Germany.
- Third-generation CD19-directed CAR construct incorporating both CD28 and 4-1BB.
- Broad spectrum of relapsed or refractory haematologic malignancies including acute lymphoblastic leukaemia, chronic lymphocytic leukaemia and lymphoma (diffuse large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma), also including patients after allogeneic stem cell transplantation.
- CD19-negative relapses might occur after CD19-directed CAR T cell therapy.
- Restriction to a single centre.

Ethics and dissemination Ethical approval and approvals from the local and federal competent authorities were granted. Trial results will be reported via peer-reviewed journals and presented at conferences and scientific meetings.

Trial registration number Eudra CT 2016-004808-60; NCT03676504; Pre-results.

INTRODUCTION

Treatment of patients with relapsed or refractory (r/r) lymphoid malignancies including acute lymphoblastic leukaemia (ALL)¹ or chronic lymphocytic leukaemia (CLL)²⁻⁵ and other non-Hodgkin's lymphoma (NHL) such as diffuse large B cell lymphoma (DLBCL),⁶ follicular lymphoma (FL)⁷ or mantle cell lymphoma (MCL)⁸ remains a challenge. For patients with such r/r CD19-positive malignancies, T cells genetically engineered to

express chimeric antigen receptors (CARs) have shown remarkable results.⁹ Clinical responses in up to 81%–89% of paediatric ALL,^{10–11} 83%–88% of adult ALL,^{12–13} 57%–71% of CLL^{14–15} and 64%–82% of NHL^{16–19} have been reported for heavily pretreated patients following CAR T cells directed against CD19 (table 1).

CARs constitute synthetic receptors composed of three domains: (1) an extracellular antigen-specific target binding domain derived from an antibody's single-chain variable fragment (scFv), (2) a hinge and transmembrane segment and (3) intracellular domain for intracellular signalling mediating activation and costimulation to the CAR-expressing T cell. First-generation CARs contain only the tyrosine-based ζ -signal-transducing subunit from the TCR/CD3 receptor complex as intracellular domain,^{20–21} whereas second-generation CARs carry costimulatory domains, for example, CD28, 4-1BB (CD137) or OX40 (CD134) adjacent to the ζ domain. Most widely, CD28 and 4-1BB costimulatory domains have been used. Both have shown to enhance CAR T cell activity and signalling and to mediate complete responses in patients with advanced CD19-positive haematologic malignancies.^{16–24} Third-generation CARs including two costimulatory molecules within their constructs, that is, CD28 and 4-1BB, have demonstrated superior proliferative capacity, a more robust survival and antitumour response *in vitro* and *in vivo* compared with second-generation CARs comprising either CD28 or 4-1BB.^{25–27} In ALL and lymphoma patients co-infused with second-generation and third-generation CD19-directed CARs, significantly superior engraftment, a 23-fold higher expansion and prolonged *in vivo* persistence of third-generation CAR T cells was reported.²⁸

In Europe, almost all clinical CAR T cell trials are industry-driven. The University Hospital of Heidelberg is currently the first institution in Germany to run an investigator-initiated trial (IIT) phase I/II CAR T cell trial (Heidelberg Chimeric Antigen receptor T cell Trial number 1 [HD-CAR-1]; EudraCT-No. 2016-004808-60; NCT03676504 (clinicaltrials.gov); federal authority No.: 3148/02; Institutional review board/Ethics Committee approval No.: AF-mu 405/2017); HD-CAR-1 clinical trial protocol version 03; date 22 June 2018; for HD-CAR-1 protocol synopsis, see online supplementary file 1). This monocentric, open-label, prospective clinical trial initiated in September 2018 treats adult patients with r/r ALL, r/r CLL or other NHL including DLBCL, FL or MCL as well as paediatric patients with r/r ALL with autologous T lymphocytes transduced with a third-generation CAR retroviral vector targeting CD19 (RV-SFG.CD19.CD28.4-1BBzeta) in-house.

METHODS AND ANALYSIS

Study design of HD-CAR-1

The study consists of three different patient strata with confirmed CD19+ (by immunohistochemistry or flow cytometry [FACS]) lymphoid disease: (1) r/r adult ALL

patients (stratum I), (2) r/r adult patients with NHL (CLL, DLBCL, FL or MCL; stratum II) and (3) r/r paediatric ALL patients (stratum III).

Autologous T lymphocytes transduced with the RV-SFG.CD19.CD28.4-1BBzeta retroviral vector (CD19.CAR T cells) are administered in three dose levels per stratum: 1×10^6 CD19.CAR T cells/m² (dose level 1 [D1]), 5×10^6 CD19.CAR T cells/m² (dose level 2 [D2]), and 20×10^6 CD19.CAR T cells/m² (dose level 3 [D3]). Three to 16 patients per stratum leading to a maximum of 48 patients will be treated in order to assess safety and maximum tolerated dose of CD19.CAR T cells (figure 1).

Following enrolment (exclusion and inclusion criteria summarised in box 1), patients undergo leukapheresis for collection of peripheral blood mononuclear cells (PBMCs). PBMCs are transduced with the RV-SFG.CD19.CD28.4-1BBzeta retroviral vector (provided by Malcolm Brenner, Baylor College of Medicine, Houston, Texas, USA) after activation with anti-CD3 and anti-CD28 antibodies (MACS GMP Pure, Miltenyi Biotec, Bergisch Gladbach, Germany) and culturing with IL-7 (10 ng/mL) and IL-15 (5 ng/mL) (CellGenix, Freiburg, Germany) at the Good Manufacturing Practice (GMP) Core Facility of the Internal Medicine V Department of the University Hospital Heidelberg. RV-SFG.CD19.CD28.4-1BBzeta carries an anti-CD19 scFv derived from the FMC63 antibody inserted to the SFG retroviral backbone.²⁹ The transmembrane domain is derived from CD28, the hinge domain from the human IgG₁-CH₂CH₃ domain³⁰ and 4-1BB is inserted between the CD28 and CD3 ζ domains (figure 2).

All patients receive lymphodepleting chemotherapy with fludarabine (flu) 30 mg/m²/day and cyclophosphamide (cyc) 500 mg/m²/day on days –4 to –2 (ie, 3 days) prior to CD19.CAR T cell infusion (defined as day 0) in an in-patient setting (figure 3).

CAR T cells are administered only to patients who have consented to study participation and fulfil the following requirements: (1) no evidence of serious infection (active infection with positive blood cultures for bacteria, fungus or virus within 48 hours of CD19.CAR T cell infusion); (2) adequate renal function defined as serum creatinine of $\leq 2 \times$ ULN or eGFR ≥ 30 mL/minute/1.73 m²; (3) no evidence of significant cardiac dysfunction; (4) no evidence of significant pulmonary dysfunction and (5) no acute neurological toxicity >grade 1 (with the exception of peripheral neuropathy). Should an event prohibit the administration of CD19.CAR T cells, the infusion must be delayed until the event resolves. If CD19.CAR T cell administration is delayed for more than 2 weeks, conditioning chemotherapy must be repeated.

Dose escalation of CD19.CAR T cells (dose 1–3; D1–D3) is performed according to a classical 3+3+4 design. Stratum I and II (adult ALL and CLL/NHL patients) recruitments are performed independently, and occurrence of dose-limiting events in one of these strata will not affect recruitment of the other one. The first cohort of three patients in stratum I and stratum II is treated with

Table 1 Published clinical trials of CD19-directed CAR T cells

Author	Number of patients/age (years)	Disease (number of patients)	Gen	Costim domain	Origin/vector	Conditioning	Infused CAR T cells	Outcome
Jensen <i>et al</i> 2010 ²¹	4 (n/a)	FL:2	I	None	auto/EP	None/flu	1–2×10 ⁹ /m ²	2 PD
Kochenderfer <i>et al</i> 2010 ⁴²	1 (n/a)	FL	II	CD28	auto/RV	cyc+flu	1–3×10 ⁸	1 PR
Savoldo <i>et al</i> 2011 ⁵⁵	6 (46–59)	NHL	I+II	None/CD28	auto/RV	None	2–20×10 ⁷ /m ²	2 SD, 4 NR
Brentjens <i>et al</i> 2011 ⁵⁶	10 (48–73)	CLL: 8 ALL: 2	II	CD28	auto/RV	None/cyc	0.3–3×10 ⁷ /kg	CLL: 1 PR, 2 SD, 1 PD, 3 NR, 1 NE ALL: 1 CR, 1 NE
Kalos <i>et al</i> 2011 ⁵⁷ /Porter <i>et al</i> 2011 ⁵⁸	3 (64–77)	CLL	II	4-1BB	auto/LV	cyc+pento/bendamrituxi	1.4×10 ⁵ /kg –1.6×10 ⁷ /kg	2 CR, 1 PR
Kochenderfer <i>et al</i> 2012 ⁵⁹	8 (47–63)	CLL: 4, FL: 3, SMZL: 1	II	CD28	auto/RV	cyc+flu + IL-2	0.3–2.8×10 ⁷ /kg	CLL: 1 CR, 2 PR, 1 SD; FL: 2 PR, 1 NE; SMZ: 1 PR
Kochenderfer <i>et al</i> 2013 ⁶⁰	10 (44–66)	CLL: 4, DLBCL: 2, MCL: 4	II	CD28	allo/RV	None	0.4–7.8×10 ⁶ /kg	CLL: 1 CR, 1 SD, 2 PD; DLBCL: 2 SD; MCL: 3 SD, 1 PR
Brentjens <i>et al</i> 2013 ²²	5 (23–66)	ALL	II	CD28	auto/RV	cyc	1.4–3.2×10 ⁸ /kg	5 CR
Cruz <i>et al</i> 2013 ⁶¹	8 (9–59)	ALL: 4 CLL: 4	II	CD28	allo/RV	None	1.5–12×10 ⁷ /m ²	3 CR, 1 PD 1 PR, 1 SD, 2 PD
Grupp <i>et al</i> 2013 ⁶²	2 (7–10)	B-ALL	II	4-1BB	auto/LV	None/cyc+eto	0.14–1.2×10 ⁷ /kg	2 CR
Maude <i>et al</i> 2014 ²³	30 (5–65)	ALL	II	4-1BB	auto/LV	Individualised	0.76–20.6×10 ⁶ /kg	27 CR, 3 NE
Davila ¹³ <i>et al</i> 2014	16 (>18)	ALL	II	CD28	auto/RV	cyc	3×10 ⁶ /kg	14 CR, 2 NR
Kochenderfer <i>et al</i> 2015 ⁶³	15 (30–68)	CLL: 4, DLBCL: 5, SMZL: 1, PMBCL: 4, LG-NHL: 1	II	CD28	auto/RV	cyc+flu	1–5×10 ⁶ /kg	CLL: 3 CR, 1 PR; DLBCL: 2 CR, 2 PR, 1 NE; SMZL: 1 PR; PMBCL: 2 CR, 1 SD, 1 NE; LG-NHL: 1 CR
Porter <i>et al</i> 2015 ¹⁴	14 (51–78)	CLL	II	4-1BB	auto/LV	cyc+flu/pento+cyc/benda	0.14–11×10 ⁶	4 CR, 4 PR, 6 NR
Lee <i>et al</i> 2015 ²⁴	21 (1–30)	ALL: 20 DLBCL: 1	II	CD28	auto/RV	cyc+flu	1–3×10 ⁶ /kg	ALL: 14 CR, 3 SD, 3 PD DLBCL: 1 PD
Brudno <i>et al</i> 2016 ⁶⁴	20 (25–68)	CLL: 5, DLBCL: 5, MCL: 5	II	CD28	allo/RV	None	0.4–8.2×10 ⁶ /kg	CLL: 1 CR, 1 PR, 1 SD, 2 PD; DLBCL: 1 CR, 3 SD, 1 PD; MCL: 1 PR, 4 SD
Dai <i>et al</i> 2015 ⁶⁵	9 (15–65)	ALL	II	4-1BB	auto/allo	none/c-MOAD	0.3–1.27×10 ⁷ /kg	ALL: 4 CR, 1 PD
Zhu <i>et al</i> 2016 ⁶⁶	2 (29–39)	ALL	II	4-1BB	auto/LV	cyc+flu	1–1.19×10 ⁶ /kg	3 CR, 3 PR, 3 PD CR: 2
Turtle <i>et al</i> 2016 ⁶⁷	32 (36–70)	NHL	II	4-1BB	auto/LV	cyc+flu / cyc/cyc+eto	0.2–20×10 ⁶ /kg	11 CR, 9 PR, 10 NR, 2 NE
Wang <i>et al</i> 2016 ⁶⁸	16 (23–75)	DLBCL: 11, MCL: 5	I+II	None/CD28	auto/LV	CAR T cells d+2 or +3 after autoSCT	2.5–20×10 ⁷	DLBCL: 8 CR, 2 PR, 1 PD; MCL: 5 CR

Continued

Table 1 Continued

Author	Number of patients/age (years)	Disease (number of patients)	Gen	Costim domain	Origin/vector	Conditioning	Infused CAR T cells	Outcome
Kebrin <i>et al</i> 2016 ⁶⁹	26 (23–61)	ALL: 17 FL: 3, DLBCL: 4, MCL: 1, HL: 1	II	CD28	auto/allo/SB	CAR T cells after autoSCT or alloSCT	Varying doses	9 CR, 2 SD, 6 PD DLBCL: 2 CR, 1 SD, 1 PD; FL: 3 CR; MCL: 1 CR; HL: 1 CR
Gardner <i>et al</i> 2017 ¹¹	43 (1–25)	ALL	II	4-1BB	auto/LV	cyc/cyc + flu	0.5–10×10 ⁶ /kg	40 CR, 2 PR, 1 PD
Locke/Kite <i>et al</i> 2017 ⁷⁰	7 (29–69)	DLBCL	II	CD28	auto/RV	cyc + flu	2×10 ⁶ /kg	4CR, 1 PR, 1 SD, 1 n/A
Hu <i>et al</i> 2017 ⁷¹	15 (7–57)	ALL	II	4-1BB	auto/LV	cyc + flu	1.1–9.8×10 ⁶ /kg	12 CR, 1 PD, 2 NE
Turtle <i>et al</i> 2017 ¹⁵	24 (40–73)	CLL	II	4-1BB	auto/LV	cyc/flu/ cyc + flu	0.2–20×10 ⁶ /kg	CR+PR: 17, 7 NR
Neelapu <i>et al</i> 2017 ¹⁸	101 (23–76)	DLBCL: 77 PBMCL or FL: 24	II	CD28	auto/RV	cyc + flu	2×10 ⁶ /kg	38 CR, 25 PR, SD 9, PD 4; NE: 1 17 CR, 3 PR, 2 SD, 1 PD, 1 NE
Schuster <i>et al</i> 2017 ¹⁷	14 (25–77) 14 (43–72)	DLBCL FL	II	4-1BB	auto/LV	Individualized	1–5×10 ⁶	6 CR, 1 PR, 7 NR 10 CR, 1 PR, 3 NR
Park <i>et al</i> 2018 ¹²	53 (23–74)	ALL	II	CD28	auto/RV	cyc/cyc + flu	1 or 3×10 ⁶ /kg	44 CR, 9 NR
Maude <i>et al</i> 2018 ¹⁰	75 (3–23)	ALL	II	4-1BB	auto/LV	cyc + flu	0.2–5.4×10 ⁶ /kg	61 CR, 6 NR, 8 NE
Li <i>et al</i> 2018 ⁷²	10 (18–59)	ALL	II	CD28/ 4-1BB	auto+allo/LV	cyc + flu	0.1–9.79×10 ⁶ /kg	6 CR, 1 PR, 3 NR
Cao ⁷³ <i>et al</i> 2018	18 (3–57)	ALL	II	4-1BB	auto/LV	cyc + flu	1×10 ⁶ /kg	14 CR, 3 NR, 1 NE
Enblad <i>et al</i> 2018 ⁵³	15 (24–71)	ALL: 4, CLL: 2, DLBCL: 6, MCL: 2, FL-Burkitt: 1	III	CD28+4-1BB	auto/RV	none/cyc + flu	2–20×10 ⁷ /m ²	ALL: 2 CR, 2 PD; CLL: 1 CR, 1 SD; DLBCL: 3 CR, 3 PD; MCL: 1 SD, 1 PD; FL-Burkitt: 1 PD
Ramos <i>et al</i> 2018 ⁵⁴	16* (16–75)	DLBCL: 11, ALL: 2, BCLL: 1, LBL: 1; CLL: 1	II+III	CD28/ CD28+4-1BB	auto/RV	cyc+flu	2–40×10 ⁶ /m ² , 0.05– 1.25×10 ⁶ /kg	DLBCL: 6 CR, 2 PR, 2 SD, 1 NR; ALL: 1 PR, 1 NR; CLL: 1 NR; BCLL: 1 CR; LBL: 1 CR

*Eleven patients with active disease; five patients (3 DLBCL, BCLL, LBL) in remission after high-dose therapy and autologous stem cell transplantation.

ALL, acute lymphoblastic leukaemia; allo, allogeneic origin; alloSCT, allogeneic stem cell transplantation; auto, autologous origin; autoSCT, autologous stem cell transplantation; BCLL, B cell lymphoma unclassified; bnda, bendamustine; CLL, chronic lymphocytic leukaemia; C-MOAD, cyclophosphamide, mitoxantrone, vindesine, cytarabine, dexmethasone; CR, complete remission; cyc, cyclophosphamide; DLBCL, diffuse large B-cell lymphoma; EP, electroporation; eto, etoposide; FL, follicular lymphoma; flu, fludarabine; Gen, CAR generation; HL, Hodgkin's lymphoma; LBL, lymphoblastic lymphoma; LG, low grade; LV, lentiviral vector; MCL, mantle cell lymphoma; n/a, not assessed; NE, not evaluable; NHL, non-Hodgkin's lymphoma; NR, no response; PD, progressive disease; pento, pentostatin; PMBCL, primary mediastinal B cell lymphoma, PR, partial response; rituxi, rituximab; RV, retroviral vector; SB, Sleeping Beauty; SD, stable disease; SMLZ, splenic marginal zone lymphoma.

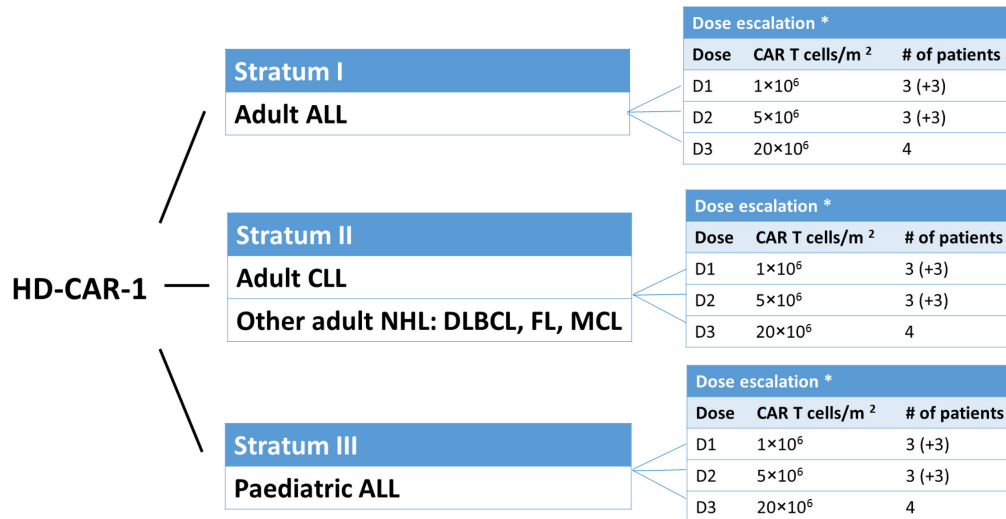


Figure 1 HD-CAR-1 treatment strata. *Dose escalation design of HD-CAR-1 is performed according to a classical 3+3+4 design. Stratum I and II (adult ALL and CLL/NHL) are recruited independently. Occurrence of dose-limiting events in one of these strata does not affect recruitment of the other one. The first cohort of three patients in stratum I and stratum II is treated with CD19.CAR T cells at dose level (D) 1. Between treatments of individual patients, a waiting period of at least 28 days is mandatory. If any of the first three patients displays DLT, three more patients are enrolled at D1. If less than three DLTs occur in this group of six patients, the study continues to D2. The same scheme is applied to progress towards D3. Initiation of stratum III (children and adolescents with r/r ALL) is performed after completion of D1 in stratum I or II without evidence of DLT in the first three patients, or with ≤ 2 DLT in the first six patients. If more than two patients display DLT at D1, D2 or D3, the DMC will be advised. An interim evaluation by the DMC is mandatory after completion of D1 and D2. ALL, acute lymphoblastic leukaemia; CLL, chronic lymphocytic leukaemia; D, dose level; DLBCL, diffuse large B cell lymphoma; DLT, dose-limiting toxicity; DMC, Data Monitoring Committee; FL, follicular lymphoma; HD-CAR-1, Heidelberg Chimeric Antigen Receptor T cell Trial number 1; MCL, mantle cell lymphoma; NHL, non-Hodgkin's lymphoma.

CD19.CAR T cells at dose level 1. Between treatments of individual patients, a waiting period of at least 28 days is mandatory. If any of the first three patients displays dose-limiting toxicity (DLT), additional three patients

are enrolled at dose level 1. If less than three DLTs occur in this group of six patients, the study continues to dose level 2. The same scheme is applied for progressing to dose level 3. Initiation of stratum III (children and adolescents with r/r ALL) occurs after completion of dose level 1 in stratum I or II without evidence of DLT in the first three patients, or with ≤ 2 DLT in the first six patients. If more than two patients display DLT at dose level 1, 2 or 3, the Data Monitoring Committee (DMC) is advised. An interim evaluation by the DMC is mandatory after completion of dose level 1 and 2. All patients who have received CD19.CAR T cells are included in the safety analysis. Patients will be excluded from the per protocol population if the patients are lost to follow-up before completion of 3 weeks post-CAR T cell administration. In these cases, new patients are recruited to fill the cohorts and assure that each cohort reaches the minimum patient numbers to finalise according to protocol. If a patient does not attend a trial visit, reasons should be clarified. All efforts have to be made to follow up the patient. In cases where a patient is lost to follow-up, reasonable efforts must be made by the study site personnel to contact the patient and determine the reason for withdrawal.

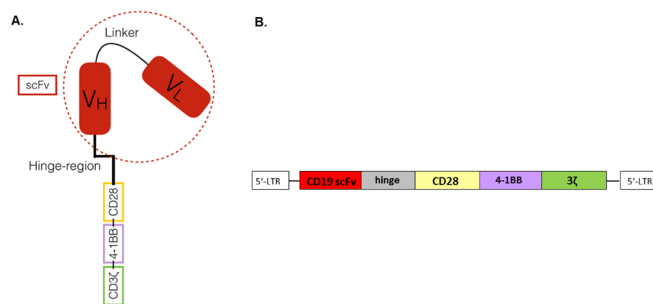


Figure 2 Structure of the HD-CAR-1 CAR. (A) Structure of the third-generation CAR construct used in the HD-CAR-1 trial. The CAR is composed of an extracellular antigen-specific scFv molecule derived from the IgG2a mouse monoclonal antibody FMC63. The scFv is attached via a flexible hinge region from the human IgG1-CH₂CH₃ domain to the CD28-derived transmembrane. This, in turn, is attached to the cytoplasmic receptor portion. The intracellular signalling domain originates from the stimulatory CD3 ζ -chain of a T cell receptor. In the third-generation HD-CAR-1 construct, costimulation is mediated by the CD28 and 4-1BB domains. (B) Linear representation of RV-SFG.CD19.CD28.4-1BBzeta.HD-CAR-1, Heidelberg Chimeric Antigen Receptor T cell Trial number 1; scFv, single-chain variable fragment.

Endpoints

Primary endpoints of HD-CAR-1 include safety and feasibility: regarding safety, frequency and grade of cytokine release syndrome (CRS), neurotoxicity (CAR-T-cell-related encephalopathy syndrome (CRES)) and other

Box 1 Inclusion and exclusion criteria of HD-CAR-1**Stratum I-II (Adults)****Inclusion criteria**

- ▶ Confirmed CD19⁺ ALL, CLL, DLBCL, FL or MCL in patients ≥18 years
- ▶ ALL: Confirmed CD19⁺ ALL (Philadelphia [Ph]⁺ and Ph⁻) by cytology and FACS AND
 - Relapsed or refractory disease (including ‘molecular relapse’ with MRD levels >10⁻³ at two occasions >2 weeks apart) with confirmed CD19-expression on malignant cells Any relapse after alloSCT (≥6 months from alloSCT at the time of CAR T cell infusion) OR
 - Any relapse failing to achieve an MRD level of <10⁻³ after ≥2 lines of treatment OR
 - Primary refractory as defined by not achieving a CR after ≥2 lines of treatment
- ▶ CLL/NHL: Confirmed CD19⁺ CLL/NHL (including CLL, DLBCL, FL or MCL)
 - CLL in need of treatment with
 1. Early relapse (within 2 years) after end of chemoimmunotherapy or chemoimmunotherapy refractoriness plus failure or intolerance of both BTK and BCL2 inhibitors OR
 2. Relapse after alloSCT, ineligible for or refractory to standard interventions (DLI, CD20 antibodies, chemoimmunotherapy)
 - DLBCL with
 1. Refractoriness to a second or later line of chemoimmunotherapy OR
 2. Relapse after autoSCT plus ineligibility for alloSCT (including refractoriness to one line of salvage chemoimmunotherapy) OR
 3. Relapse after alloSCT
 - FL in need of treatment with
 1. Relapse <2 years after chemoimmunotherapy AND ineligibility for or failure of autoSCT AND ineligibility for or failure of idelalisib OR
 2. Relapse after alloSCT, ineligible for or refractory to standard interventions (donor lymphocyte infusions, CD20 antibodies, chemoimmunotherapy)
 - MCL with
 1. Relapse after standard first-line therapy AND ineligibility for or failure to BTKi salvage therapy OR
 2. Relapse after alloSCT AND ineligibility for or failure to BTKi salvage therapy
- ▶ Measurable disease/MRD at the time of enrollment
- ▶ Life expectancy ≥12 weeks
- ▶ ECOG performance status ≤2 at the time of screening
 - Adequate organ function Renal function defined as serum creatinine of ≤2 times ULN or eGFR ≥30 mL/minute/1.73 m²
 - Liver function defined as ALT ≤5 times the ULN for the respective age
 - Bilirubin ≤2.0 mg/dL with the exception of patients with hyperbilirubinemia explained by Gilbert-Meulengracht syndrome (may be included if total bilirubin is ≤3.0x ULN and direct bilirubin ≤1.5x ULN) or extrahepatic disease (eg, chronic haemolytic anaemia)
- ▶
 - Minimum level of pulmonary reserve defined as ≤grade 1 dyspnoea and pulse oxygenation >90% on room air
 - Haemodynamic stability and LVEF ≥40% as confirmed by echocardiogram
 - ANC ≥500/mm³
 - ALC ≥100/mm³

- ▶ Women of childbearing potential (defined as all women physiologically capable of becoming pregnant) and all male participants must agree to use highly effective methods of contraception for 1 year following CD19.CAR T cell therapy
- ▶ Ability to understand the nature of the trial and the trial-related procedures
- ▶ Written informed consent must be obtained prior to any screening procedures

Exclusion criteria

- ▶ The following medications are excluded:
 - Immunosuppressive medication with the exception of ≤30 mg prednisolone/d or equivalent at the time of CAR T cell transfusion
 - Bridging/maintenance therapy including chemotherapy and immunotherapy must be stopped ≥2 weeks prior to leukapheresis, but can be continued between leukapheresis and lymphodepletion
- ▶ Intrathecal chemotherapy is possible at any time, but not during lymphodepletion until 14 days after CD19.CAR T cell transfusion
- ▶ Any DLI must be completed >6 weeks prior to CD19.CAR T cell infusion
- ▶ Florid/acute or chronic GvHD
- ▶ Uncontrolled active hepatitis B or C
- ▶ HIV-positivity
- ▶ Uncontrolled acute life-threatening bacterial, viral or fungal infection
- ▶ Severe concomitant disease (eg, uncontrolled arterial hypertension, heart failure NYHA III–IV, uncontrolled diabetes mellitus and uncontrolled hyperlipidaemia)
- ▶ Unstable angina and/or myocardial infarction within 3 months prior to screening
- ▶ Any previous or concurrent malignancy
- ▶ The following exceptions do not constitute exclusion criteria:
 - Adequately treated basal cell or squamous cell carcinoma (adequate wound healing is required prior to study entry)
 - In situ carcinoma of the cervix or breast, treated curatively without evidence of recurrence ≥3 years prior to the study

Continued

Box 1 Continued

- CLL or FL transformed into an aggressive B cell lymphoma
- A primary malignancy which is in complete remission for ≥ 5 years
- ▶ Pregnant or nursing (lactating) women
- ▶ Intolerance to the excipients of the cell product
- ▶ Active CNS involvement in ALL patients at the time of screening is not an exclusion criterion, but patients with CNS 3 status at clinical screening (d-14) are not eligible for CD19.CAR T cell transfusion
- ▶ Participation in another clinical trial at the time of screening

Stratum III (Children and adolescents with ALL)**Inclusion criteria**

- ▶ Age of >3 years until <18 years at the time of screening
- ▶ CD19⁺ ALL (Ph⁺ and Ph⁻) confirmed by cytology and FACS AND
- ▶ Relapsed or refractory disease (including 'molecular relapse' with PCR-MRD $>10^{-3}$ at two occasions >2 weeks apart) with confirmed CD19-expression on malignant cells
 - Any relapse after alloSCT (≥ 6 months from alloSCT at the time of CAR T cell infusion) OR
 - Any relapse failing to achieve an MRD level of $<10^{-3}$ after ≥ 2 lines of treatment OR
 - Primary refractory as defined by not achieving a CR after ≥ 2 lines of treatment
- ▶ Measurable disease/MRD at the time of enrollment
- ▶ Life expectancy ≥ 12 weeks
- ▶ ECOG performance status ≤ 2 (age ≥ 16 years) or Lansky performance status ≥ 50 (age <16 years) at the time of screening
- ▶ Adequate organ function
 - Renal function defined as serum creatinine clearance ≥ 30 mL/minute/1.73 m²
 - Liver function defined as
 - ALT ≤ 5 times the ULN for the respective age
 - Bilirubin ≤ 2.0 mg/dL with the exception of patients with hyperbilirubinaemia explained by Gilbert-Meulengracht syndrome or extrahepatic disease (eg, chronic haemolytic anaemia)
 - Minimum level of pulmonary reserve defined as \leq grade 1 dyspnoea and pulse oxygenation $>90\%$ on room air
 - Haemodynamic stability and LVEF $\geq 40\%$ or shortening fraction $>29\%$ as confirmed by echocardiogram
 - ANC $\geq 500/\text{mm}^3$
 - ALC $\geq 100/\text{mm}^3$
- ▶ Women of childbearing potential (defined as all women physiologically capable of becoming pregnant) and postpubertal male participants must agree to use highly effective methods of contraception for 1 year following CD19.CAR T cell therapy
- ▶ Written informed consent of the study patient and/or the legal representative must be obtained prior to any screening procedures

Exclusion criteria

- ▶ The following medications are excluded:
 - Immunosuppressive medication with the exception of <0.5 mg/day*kg BW prednisolone-equivalent at the time of CD19.CAR T cell transfusion
 - Bridging/maintenance therapy including chemotherapy and immunotherapy must be stopped ≥ 2 weeks prior to leukapheresis, but can be continued between leukapheresis and lymphodepletion
- ▶ Intrathecal chemotherapy is possible at any time, but not during lymphodepletion until 14 days after CD19.CAR T cell transfusion
- ▶ Any DLI must be completed >6 weeks prior to CD19.CAR T cell infusion
- ▶ Florid/acute or chronic GvHD
- ▶ Uncontrolled active hepatitis B or C
- ▶ HIV-positivity
- ▶ Uncontrolled acute life-threatening bacterial, viral or fungal infection
- ▶ Severe concomitant disease (eg, any life-limiting genetic disorder). Patients with down syndrome will not be excluded
- ▶ Any previous or concurrent malignancy
- ▶ The following exceptions do not constitute exclusion criteria:
 - Lymphoblastic lymphoma transformed into a CD19⁺ acute lymphoblastic leukaemia
 - A primary malignancy which is in complete remission for ≥ 5 years
- ▶ Pregnant or nursing (lactating) women
- ▶ Intolerance to the excipients of the cell product
- ▶ Active CNS involvement at the time of screening is not an exclusion criterion, but patients with CNS 3 status at clinical screening (d-14) are not eligible for CD19.CAR T cell transfusion
- ▶ Participation in another clinical trial at the time of screening

ALC, absolute lymphocyte count; ALL, acute lymphoblastic leukaemia; alloSCT, allogeneic stem cell transplantation; ALT, alanine aminotransferase; ANC, absolute neutrophil count; autoSCT, autologous stem cell transplantation; CLL, chronic lymphocytic leukaemia; CNS, central nervous system; CR, complete remission; DLBCL, diffuse large B-cell lymphoma; DLI, donor lymphocyte infusions; ECOG, Eastern Cooperative Oncology Group; eGFR, estimated glomerular filtration rate; FACS, flow cytometry; FL, follicular lymphoma; GvHD, Graft-versus-Host disease; HD-CAR-1, Heidelberg Chimeric Antigen Receptor T cell Trial number 1; LVEF, left ventricular ejection fraction; MCL, mantle cell lymphoma; MRD, minimal residual disease; NHL, non-Hodgkin's lymphoma; ULN, upper limit of normal.

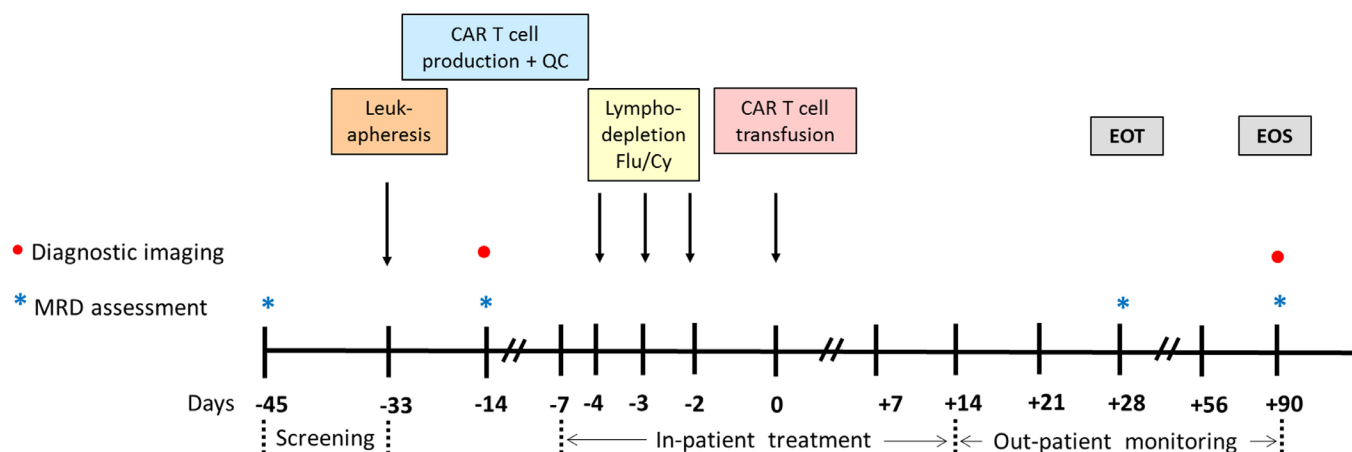


Figure 3 HD-CAR-1 clinical trial: general time schedule. After inclusion, patients undergo leukapheresis followed by CAR T cell production. After clearance of QC of the CD19.CAR T cell product, patients receive a preconditioning therapy consisting of fludarabine (flu) 30 mg/m²/day and cyclophosphamide (cy) 500 mg/m²/day (day -4 to day -2) followed by a rest day on day -1. Patients receive CD19.CAR T cells intravenously on treatment day 0. CD19.CAR T cell infusion is followed by at least 14 days of in-patient observation. This period is followed by out-patient monitoring with EOT on day 28 and EOS on day 90 after CD19.CAR T cell administration. EOS, end-of-study; EOT, end-of-treatment; HD-CAR-1, Heidelberg Chimeric Antigen Receptor T cell Trial number 1; MRD, minimal residual disease; QC, quality control.

adverse events (AEs) will be assessed. CRS will be graded according to adapted grading criteria proposed by Lee *et al* 2014³¹ and Davila *et al* 2014¹³ (online supplementary table 1.A and B). Grading of CRES will be assessed according to the Common Terminology Criteria for Adverse Events (CTCAEV.5.0) and an orientating 10-point examination (online supplementary table 1.C). Management of CRS and CRES is summarised in online supplementary figure 1 and 2. Other toxicities are assessed according to CTCAEV.5.0.

DLT is defined as any AE that is not (1) pre-existing or due to (2) the underlying malignancy. CRS and neurotoxicity are considered as DLT if \geq grade 4. Non-haematologic DLT is any grade 3 or higher toxicity (according to CTCAEV.5.0) occurring within 90 days after CD19.CAR T cell administration. Haematologic DLTs are defined as any grade 4 (except lymphopaenia) toxicity (according to CTCAEV.5.0) lasting $>$ 30 days. Patients with evidence of bone marrow disease are not evaluable for haematologic DLT. The following toxicities are not considered DLTs: laboratory tumour lysis syndrome (TLS) and grade 1 clinical TLS according to the Cairo and Bishop classification,³² hypoalbuminaemia, transient ($<$ 72 hours) grade 4 hepatic enzyme abnormality and/or grade 3 or 4 fever or neutropenic fever.

With regards to feasibility, successful transduction and manufacturing of respective doses of CD19.CAR T cells are assessed. Manufacturing failure is considered if $<$ 50% of the intended CD19.CAR T cell dose could be produced. CD19.CAR T cells are not administered in case of (1) serious infection, (2) impaired renal, cardiac or pulmonary dysfunction or (3) signs of neurological toxicity $>$ grade 1 (with the exception of peripheral neuropathy).

Secondary endpoints include monitoring of survival and function of CD19.CAR T cells in treated patients. Detection of CD19.CAR T cells will be performed by quantitative PCR. Additionally, evaluation of antitumour efficacy of CD19.CAR T cells in patients at day 90 (end-of-study [EOS]) after CD19.CAR T cell infusion (overall response rate, complete remission [CR], partial response [PR]) is assessed. Furthermore, time to response (at least PR), duration of overall response (DOR), progression-free survival (PFS) and overall survival (OS) after CD19.CAR T cell transfusion are determined. Statistical analysis is performed via descriptive methods: for primary endpoint analysis, summary tables display the number of patients observed with AEs according to CTCAE, as well as frequency and grade of CRS and CRES. All secondary endpoint variables are analysed using explorative and mainly descriptive methods. Summary statistics for categorical variables will include frequency counts and percentages.

Data collection, handling, management and monitoring

Patients are evaluated as outlined in the study calendar (table 2). All data are documented on case report files (CRFs). Patient data are documented pseudonymously. The investigator, or a designated representative, completes the CRF forms as soon as possible after the information is collected. Explanation should be given for all missing data. All entries in the CRF must be verifiable by source documents. The investigator is responsible for ensuring that all forms of the CRF are completed correctly and that entries can be verified against source data. The CRF/database must contain a full audit trail, in order to make all changes applied to the data after their first entry reproducible. To ensure data quality, regular monitoring

Table 2 Schedule of study visits

Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Screening	Inclusion	Leuk-apheresis	Clinical screening	Hospitalisation+ chemotherapy	Application of CAR T cells	Post-CAR visit 1	Post-CAR visit 2	Post-CAR visit 3	Post-CAR visit 4	Post-CAR visit 5	Post-CAR visit 6	EOT	Follow-up visit 1	EOS
Days (d)	d-45±3‡	d-40±3‡	d-33±3‡	d-14±3‡	d-6 to d-2	0	d+1	d+2	d+3	d+7	d+14	d+21	d+28±3	d+56±3	d+90±3
Screening															
Informed consent	x														
Screening ID	x														
Patient ID		x													
PBMC collection															
			x												
Inclusion/exclusion criteria review	x														
Infectiology testing§															
	x		x												
Turnour assessment															
Expression CD19*	x											x			
Diagnostic imaging¶				x											x
Safety assessment															
Medical history	x														
PE	x		x	x	x	x	x	x	x	x	x	x	x	x	x
12-lead ECG	x			x	x (d-6 only)		x			x		x			
Documentation of concomitant medication	x		x	x	x	x	x	x	x	x	x	x	x	x	x
CTC assessment			x	x	x	x	x	x	x	x	x	x	x	x	x
QOL (adults only)	x												x		
Laboratory assessment															
Clinical chemistry**	x		x	x	x (d-6,-4,-2)	x	x	x	x	x	x	x	x	x	x
Haematology††	x		x	x	x	x	x	x	x	x	x	x	x	x	x
Urine analysis	x														
Thyroid parameters‡‡	x				x (only d-6)								x		
Serum pregnancy test	x			x	x										
MRD assessment in PB and/or BM§§	x			x									x		x
CSF cytology¶¶	x			x									x¶¶		
IL-6 assessment				x		x	x	x	x	x	x	x	x	x	x
Immunoglobulin assessment***				x									x	x	x

Continued

Table 2 Continued

Visit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
	Screening		Inclusion	Leuk-apheresis	Clinical screening	Hospitalisation+ chemotherapy	Application of CAR T cells	Post-CAR visit 1	Post-CAR visit 2	Post-CAR visit 3	Post-CAR visit 4	Post-CAR visit 5	Post-CAR visit 6	EOT	Follow-up visit 1	EOS
Days (d)	d-45±3‡	d-40 ±3‡	d-33 ±3‡	d-14±3‡	d-6 to d-2	0	d+1	d+2	d+3	d+7	d+14	d+21	d+28±3	d+56±3	d+90±3	
<i>Study treatment</i>																
CAR TC transfusion						x										
Immunogenicity assessment																
40 mL EDTA† blood and PBMC isolation‡‡	x		x\$\$\$	x	x (only d-6)	x	x	x	x	x	x	x	x	x	x	x
8 mL serum	x		x	x	x (only d-6)	x	x	x	x	x	x	x	x	x	x	x

*CD19 expression confirmed by FACS or immunohistochemistry.

‡ Minimum period between screening/inclusion/leukapheresis and CD19 CAR TC infusion is indicated. Upon clinical need, this time may be prolonged to apply antileukaemic treatment.

§ Infection testing: HIV, HCV, HBV, Treponema pallidum, Toxoplasmosis.

¶ Performed in case of CLL/NHL, but not in ALL patients (standard treatment according to local standards).

** Clinical chemistry: electrolytes (Na, K, Ca), creatinine, urea, GFR (according to CKD-EPI), ASAT, ALAT, GGT, total bilirubin, LDH, CRP, ferritin.

†† Haematology: blood count, differential count, platelets, clotting parameters (Quick, INR, aPTT).

‡‡ Thyroid parameters: TSH; if TSH is deviant, levels of free T3 and T4 will be measured.

\$\$\$ MRD assessment: according to disease and standard diagnostic procedures (eg, MRD-flow, bcr/abi-PCR and IGHV RQ-PCR).

§§ Only, if prior CNS involvement.

*** Immunoglobulin assessment: IgG, IgM, IgA.

‡‡‡ Max. 2 mL/kg BW for children and adolescents; from this material, CD19 CAR T cell frequency will be assessed by FACS and qPCR as well as CD19+ B cell frequency by FACS.

\$\$\$ Aliquot from leukapheresis product.

ALAT, alanine transaminase; ALL, acute lymphoblastic leukaemia; aPTT, activated partial thromboplastin time; ASAT, aspartate amino transferase; BM, bone marrow; BW, body weight; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukaemia; CNS, central nervous system; CRP, C-reactive protein; CSF, cerebral spinal fluid; CTC, common toxicological criteria; EDTA, ethylenediamine tetraacetate; EOS, end-of-study; EOT, end-of-treatment; FACS, flow cytometry; GFR, glomerular filtration rate; GGT, gamma-glutamyl transferase; HBV, hepatitis B virus; HCV, hepatitis C virus; ID, identification number; IGHV-RQ PCR, immunoglobulin heavy chain gene real-time quantitative PCR; IL-6, interleukin-6; INR, international normalized ratio; LDH, lactate dehydrogenase; MRD, minimal residual disease; NHL, non-Hodgkin's lymphoma; PB, peripheral blood; PBMC, peripheral blood mononuclear cells; PE, physical examination (including vital signs, height, weight); PM, precision medicine; QOL, quality of life; qPCR, quantitative real-time PCR; TSH, thyroid stimulating hormone.

of the data entry will be done at site by an independent clinical monitor (CONVIDIA clinical research GmbH). The monitor surveys completeness, validity and plausibility of data. Missing data or inconsistencies will be reported back to the trial centre and have to be clarified by the responsible investigator prior to database lock. If no further corrections are to be made in the database after completion of the trial, it will be declared locked and used for statistical analysis.

The clinical study centre of the Medizinische Klinik V of the University Hospital Heidelberg is responsible for archiving the Trial Master File (TMF) including the trial protocol, CRFs, written opinions on the protocol and procedures, final reports, audit certificates and all other relevant documents in accordance to the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Harmonized Tripartite Guideline on Good Clinical Practice (GCP) (as effective by 14 June 2017).

Serious adverse event (SAE) must be reported to the clinical monitor within 24 hours after the SAE becomes known using a defined SAE form. If new information on the risk-to-benefit ratio is obtained and safety concerns arise, the sponsor reserves the right to interrupt or terminate the trial. The investigators can recommend interruption or termination of the study or of treatment arms based on the results of the intermittent SAE evaluation or of accumulating information on abovementioned reasons. The ethics committee (EC) and the competent authorities will be informed about the premature closure of the trial or one of the treatment arms. Furthermore, the EC and competent authorities themselves may decide to stop or suspend the trial. All involved investigators have to be informed immediately about a cessation/suspension of the trial. The decision is binding to the trial centre and involved investigators. According to §40 German Drug Law (AMG), an insurance policy is effective (Ecclesia GmbH) covering for harm or injuries caused to participating patients by the trial and arising out of this research.

Data monitoring is performed by on-site and off-site visits from the independent clinical monitor. The investigator must allow the monitor to verify all essential documents and must provide support at all times to the monitor. Regulatory authorities and/or auditors authorised by the sponsor may request access to all source documents, CRFs and other trial documentation. DMC acts according to the Data Monitoring Charter. DMC meets periodically to review summarised and individual patients data related to safety, data integrity and overall conduct of the trial and review specific interim analyses for safety and/or efficacy, as appropriate. Additionally, DMC provides recommendations to continue as originally planned, change or terminate the trial depending on these analyses, communicate other recommendations or concerns as appropriate and operate according to the procedures described in the Data Monitoring Charter. DMC members defined in the HD-CAR-1 clinical trial

protocol and Data Monitoring Charter are individuals who are impartial and independent of the investigators and the sponsor and who have no financial, scientific or other conflict of interest with the study. As soon as data regarding 1 month PFS for the first three patients are available, interim analyses by the DMC is performed. Results are disseminated inside or outside the study team.

Confidentiality and access to data

Confidentiality of trial data is provided according to the European Datenschutz-Grundverordnung (DSGVO) and the German Bundesdatenschutzgesetz. The data obtained in the course of the trial will be treated pursuant to the Federal Data Protection Law (Bundesdatenschutzgesetz, BDSG). During the clinical trial, enrolled patients are identified solely by means of their individual identification code (subject number, randomisation number). Trial findings stored in a computer are stored in accordance with local data protection law and will be handled with strictest confidentiality. The appropriate regulations of local data legislation are fulfilled in its entirety. The principal investigator and the study physicians will directly and personally obtain consent and assent from enrolled patients. Each enrolled patient consents via written informed consent to allow access to his/her original medical records for trial-related monitoring, audit and regulatory inspection. Authorised persons (clinical monitors, auditors, inspector) may inspect patient-related data collected during the trial ensuring the data protection law. The investigator maintains a patient identification list to enable records to be identified. Clinical trial protocol, CRFs, other results forms and laboratory data are not disclosed to third parties. Staffs of the investigators involved in the trial are bound by this agreement.

Protocol amendments

All planned substantial changes to the clinical trial protocol are submitted to the EC and the competent authority in writing as protocol amendments. They have to be signed by the sponsor and approved by the EC and the competent authority.

Patient and public involvement

Patients and public were not involved in research preceding this study. They were not involved in the design, recruitment or conduction of this trial.

Ethics and dissemination

HD-CAR-1 clinical trial will be conducted according to the principles of the Declaration of Helsinki (2008). Written informed consent will be taken from all participants, and confidentiality and anonymity of patients granted in accordance to German general national regulatory requirements, that is, the Bundesdatenschutzgesetz (BDSG). After completion of the trial, HD-CAR-1 participants, physicians, the public and other relevant groups will be informed of the study results via peer-reviewed journal publications, presentation of results at relevant conferences as well as scientific meetings.

DISCUSSION

Why do we need CARs?

Treatment of B cell malignancies has significantly improved in the last decades. Children and adolescents diagnosed with B-ALL and treated with conventional treatment display cure rates of about 90%.³³ However, prognosis declines dramatically in adult ALL or r/r childhood ALL: adult ALL patients display OS rates of <50%³⁴ and paediatric patients with refractory ALL not reaching CR with negative minimal residual disease (MRD) have survival rates of <10%.³⁵

Treatment for patients with CLL and other NHL has also improved, particularly due to the B cell receptor inhibitors ibrutinib (BTK inhibitor) and idelalisib (PI3K inhibitor) as well as the BCL2 inhibitor venetoclax.^{36–39} However, CLL patients with failure to ibrutinib³ or idelalisib⁴⁰ and ineligible or refractory to venetoclax² have a very poor outcome. DLBCL patients with resistance to primary and salvage chemotherapy or relapse within 12 months following autologous SCT display median OS of only 6 months.⁶ Also, patients with FL refractory to chemoimmunotherapy and disease progression under idelalisib have 2-year median survival rates of only 22%.⁷ With regards to relapsed MCL, treatment is often ineffective, leading to short survival.⁴¹

After almost one decade of clinical experience with CD19-directed CARs,^{21 42} CAR T cells have become an available treatment option for patients with CD19-positive lymphoid malignancies. Second-generation CAR T cells targeting CD19 have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in October 2017 and August 2018, respectively: axicabtagene ciloleucel (Yescarta) carrying CD28 as a costimulatory domain is approved for patients with DLBCL, transformed FL and primary mediastinal B cell lymphoma who have not responded to or who have relapsed after at least two treatment lines. Tisagenlecleucel (Kymriah) carrying 4-1BB as a costimulatory domain is approved for patients up to 25 years of age with ALL that is refractory or in second or later relapse as well as adult patients with r/r DLBCL after two or more lines of systemic therapy.

How to speed up CAR T cell therapy in Europe?

Axicabtagene ciloleucel and tisagenlecleucel were recently approved by the EMA. However, it will still take some time until all logistic and legal issues will be cleared. All of these products are currently produced outside of Europe and therefore both leukapheresis and CAR T cell products need to be cryopreserved and shipped to the manufacturing facility overseas and back. Moreover, >90% of CAR T cell trials are performed outside Europe, that is, in the USA and the P.R. China.⁴³ This disparity in the geographic location is due to (1) lacking access to GMP facilities given that CAR T cell manufacturing in the European Union (EU) requires GMP compliance on an industrial level, (2) complex authorisation processes and (3) differences in the respective regulatory requirements within individual EU states. In Germany, at present only four clinical CAR T cell trials are registered,

all being industry-driven (NCT02445222 [Novartis], NCT02348216 [Kite/Gilead], NCT03484702 [Celgene] and NCT02445248 [Novartis]) (clinicaltrials.gov search on 2 September 2018 for terms 'CAR T cells+Germany').

Preparation for approval of the current HD-CAR-1 trial took approximately 2.5 years. Whereas in the USA, the FDA is the only authority regulating CAR T cell manufacturing as well as clinical administration, CAR T cell therapy in Germany involves several regulatory institutions: federal (Paul-Ehrlich Institute [PEI]) as well as local (Regierungspraesidium [RP]) regulatory authorities authorise and survey clinical trials and CAR T cell manufacturing in accordance with GMP standards. Moreover, the local EC monitors patient safety. Although clinical trial regulation for Europe has been updated in 2014 and the authorisation process is aimed to be harmonised in the near future, current authorisation requires simultaneous submission of applications to the distinct authorities: for HD-CAR-1, initial applications were submitted in September 2016 to the PEI and the EC of the University of Heidelberg. Approval from the EC, the PEI competent authority and the RP local authority were granted in October 2017, September and August 2018, respectively. HD-CAR-1 was initiated on 7 September 2018.

Why do we need this trial?

The majority of clinical CAR T cell trials have used second-generation CAR constructs incorporating either CD28 or 4-1BB. CD28 has been associated with robust CAR T cell expansion, rapid tumour elimination *in vitro*^{44–46} and persistence up to 3 months in treated patients.^{13 22 24} 4-1BB, in turn, has displayed longer *in vitro*^{47 48} and *in vivo* persistence^{49 50} when compared with CD28, has been associated with reduced CAR T cell exhaustion⁵¹ and been detectable for more than 5 years in patients treated with CD19-directed CAR T cells.^{14 23} It has been shown that 4-1BB costimulation promotes central memory CAR T cells, diminishes exhaustion and mediates a metabolic profile that results in enhanced CAR T cells persistence.^{44 51} In contrast, CD28 costimulation results in differentiation into effector memory CAR T cells with short-lived glycolysis-based metabolism.⁴⁴ Based on these distinct characteristics, third-generation CARs comprising both these elements might display short-term efficacy (CD28) as well as long-term persistence (4-1BB). Nonetheless, also reduced efficacy and increased levels of apoptosis have been related to third-generation CAR T cells,⁵² highlighting the need for further clinical evaluation.

Turtle *et al*¹⁶ demonstrated that chemotherapy with flu and cyc preceding CAR T cell administration improved the outcome of treated patients. In contrast to previous trials, flu and cyc conditioning will be consequently incorporated within the HD-CAR-1 trial (see figure 3). Furthermore, most of the trials including the pivotal CD19-directed CAR T cell trials JULIET and ZUMA-1/–2¹⁸ excluded patients after allogeneic stem cell transplantation (alloSCT). HD-CAR-1 allows treatment of these

patients since this patient population is particularly in need of novel treatment options.

Two clinical trials using third-generation CARs targeting CD19 have been recently published.^{53 54} The first trial included heavily pretreated leukaemia and lymphoma patients displaying significant comorbidities. Following treatment with CAR T cells comprising CD28 and 4-1BB costimulatory domains, responses could be achieved in 40% of these patients.⁵³ Several conceptual differences of this trial and our own study initiated in September 2018 exist, that is: (1) only 70% of patients received lympho-depleting chemotherapy consisting of low-dose cyclophosphamide, (2) CAR T cell manufacturing differs in terms of stimulation of CAR T cell culture (IL-2 vs IL-7/-15), (3) only adult patients were included, whereas our trial will include paediatric patients also.

The second trial investigated the benefit of third-generation CAR T cells administering simultaneously second-generation (containing only CD28 as costimulatory domain) and third-generation CAR T cells (CD28 and 4-1BB; identical to the HD-CAR-1 vector) to NHL patients. Responses in patients with r/r disease were achieved in 54% of treated patients. Compared with cells transduced with the second-generation CAR vector, third-generation CAR T cells displayed superior expansion and longer persistence, particularly in patients with low disease burden and low levels of circulating B cells.⁵⁴ Nonetheless, the number of patients treated with third-generation CAR T cells remains low, and further studies are required for detailed evaluation of third-generation CD19-directed CAR T cells.

HD-CAR-1 of the University Hospital Heidelberg is the first CAR T cell IIT in Germany and evaluates third-generation CD19.CAR T cell administration to adult and paediatric patients with r/r ALL as well as patients with r/r CLL or other NHL. Given that leukapheresis, CD19.CAR T cell manufacturing, administration, patient monitoring and follow-up are performed in-house, independence of transport systems and production sites outside the University Hospital Heidelberg or even Europe is provided. Additionally, close interaction with the PEI, RP and academic discussion and exchange support and promote treatment with CAR T cells and other novel cell therapies in Germany and Europe.

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Contributors MS is the principle investigator (PI), PD is the deputy PI of the HD-CAR-1 clinical trial. MS, M-LS, PD, LS, AS, JK, ADH, CM-T, PW, AKul and SH reviewed the protocol of the trial. MS, M-LS, AS, LS, BN, JK, AH-K, AKul and PD were involved in the process of obtaining approval for the clinical trial from the ethical committee and the competent authority. MS, AS, BN, UG, AH-K, BM and AKul worked on the Good Manufacturing Practice (GMP) CD19.CAR T cell generation protocol and its approval from the local authority for obtaining the manufacturing license. M-LS, LS, MS and PD wrote the study protocol. M-LS wrote the primary manuscript. All authors critically reviewed the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Ethical approval and approvals from the local and federal competent authorities were granted. HD-CAR-1 trial protocol received Institutional Review Board approval from the EC of the University of Heidelberg in October 2017 (AFmu-405/2017).

Provenance and peer review Not commissioned; externally peer reviewed.

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