Personalised multipeptide-based T-cell activator for chronic lymphocytic leukaemia: an open-label, single-centre, phase 1 study

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Summary

Background Therapeutic T-cell activation to induce tumour-specific immune responses promises sustainable cancer control. However, this treatment is not yet widely used because of the challenges of personalised drug design and a shortage of mutation-derived neoepitopes. This study aimed to evaluate the immunogenicity, safety, and toxicity of iTAC-XS15-CLL01, a personalised warehouse-based multipeptide T-cell activator combined with the Toll-like receptor 1/2 ligand XS15, in patients with chronic lymphocytic leukaemia who were undergoing Bruton's tyrosine kinase inhibitor (BTKi)-based regimes.

Methods This open-label, single-centre, phase 1 study, conducted in Germany, enrolled patients aged 18 years or older who had chronic lymphocytic leukaemia with an Eastern Cooperative Oncology Group score of 2 or lower and were due to receive a BTKi-based regimen either as monotherapy or in combination (eg, with anti-CD20). The patients' HLA allotype had to match at least one of the corresponding HLA alleles of peptides included in the peptide warehouse. To begin treatment with iTAC-XS15-CLL01, patients had to have reached at least partial remission with remaining minimal residual disease after 6–8 months of BTKi therapy. iTAC-XS15-CLL01 comprised eight chronic lymphocytic leukaemia-specific peptides, selected for the individual patient from a peptide warehouse on the basis of HLA allotyping and immunopeptidomics. Participants received three monthly doses of iTAC-XS15-CLL01 (consisting of 300 μg of each peptide plus 50 μg XS15, emulsified in Montanide ISA 51 VG), injected subcutaneously. The primary endpoints were induction of a T-cell response after application of iTAC-XS15-CLL01 compared with baseline, as assessed with IFNγ ELISpot assays, and the frequency and severity of adverse events from the first application of iTAC-XS15-CLL01 to end of treatment. All analyses were done per protocol. This study was registered with ClinicalTrials.gov (NCT04688385) and is now closed.

Findings Between Jan 21, 2021, and July 7, 2023, 30 patients with chronic lymphocytic leukaemia were screened, and 20 were recruited to enter the iTAC-XS15-CLL01 treatment phase and followed up for 6 months. All patients were of White ethnicity; six (30%) patients were female, and 14 (70%) were male. Median age was 56·5 years (IQR 49·5–65·5). The most common grade 3 adverse events were injection-site erythema (three [15%] of 20 patients), granuloma (two [10%] of 20), and ulceration (one [5%] of 20). There were no grade 4 adverse events, treatment-related serious adverse events, or deaths. T-cell responses targeting multiple peptides were induced in 19 (95% [95% CI 75·1–99·9]) of 20 patients at end of treatment and persisted in 16 (84%) of 19 at 6 months follow-up, with the intensity of responses increasing until end of study.

Interpretation Our findings indicate that iTAC-XS15-CLL01 could be a potent immunotherapeutic agent in patients with chronic lymphocytic leukaemia and should be further evaluated in phase 2 trials.

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Introduction

Chronic lymphocytic leukaemia is the most common leukaemia, with global incidence increasing by more than 150% since 1990.[1] Over the past decade, treatment options have improved substantially, with targeted therapies, including Bruton tyrosine kinase inhibitors (BTKi), now a standard of care.[2] Most available therapies achieve only partial remissions, and none of the currently available therapies enables a cure. As a result, continuous treatment is needed, which leads to a high risk of treatment resistance and side-effects in patients.[3] This scenario creates a high medical need for well-tolerated treatment approaches to eliminate the treatment-resistant residual chronic lymphocytic

Research in context

Evidence before this study

We searched PubMed and ClinicalTrials.gov for original, peer-reviewed studies from trial inception (Nov 15, 2019) to March 4, 2020, using the search terms "immunopeptidome", "ligandome", "peptide-based therapy" and "cancer", without language restrictions. We reviewed key journals (*Nature*, *The Lancet*, *Science*, and *Journal of Clinical Oncology*) but found no trial evaluating immunopeptidome-guided peptide-based T-cell activators in any cancer. In previous work, we had established an immunopeptidome-guided warehouse-based T-cell activator platform, combining naturally presented HLA class I and II T-cell epitopes for various cancer entities, including chronic lymphocytic leukaemia, with the novel Toll-like receptor 1/2 ligand XS15 emulsified in the adjuvant Montanide. We conducted two trials proving the safety and excellent immunogenicity of XS15-adjuvanted peptide-based T-cell activators using SARS-CoV-2-derived T-cell epitopes in healthy volunteers and patients with cancer and provided the first evidence for the clinical activity of such activators in combating cancer. We combined the expertise from our preclinical work and previous trials to develop iTAC-XS15-CLL01, a warehouse-based personalised T-cell activator for patients with chronic lymphocytic leukaemia, and designed a trial to evaluate its safety and immunogenicity, along with early signs of clinical activity.

Added value of this study

In this trial, we characterise a novel T-cell activator for patients with chronic lymphocytic leukaemia as a safe and potent immunotherapeutic agent. Additionally, this study introduces an option for personalised drug design in the treatment of chronic lymphocytic leukaemia. The trial establishes a new workflow for building immunopeptidome-defined non-mutated peptide warehouses for the fast-track personalised design of T-cell activator therapies in cancer.

Implications of all the available evidence

The safety, immunogenicity, and early clinical activity results of this trial show that peptide-based T-cell activators offer a promising, low side-effect approach to drive immunity in chronic lymphocytic leukaemia. Further evaluation of these activators in randomised phase 2 trials is warranted, as well as the expansion of this workflow to design personalised therapies for other cancer entities.

leukaemia cells (measurable residual disease [MRD]) that mediate disease relapse,[4] enabling long-lasting remissions. Data on graft-versus-leukaemia effects after allogeneic stem cell transplantation[5] point to T-cell-based immune control of the disease, which could be induced by immunotherapeutic approaches. However, the immune deficiency associated with chronic lymphocytic leukaemia[6] and the low mutational burden of this disease—which results in a scarcity of neoepitopes acting as targets for T cells—limit antitumor immunity and, thus, also the clinical efficacy of immunotherapies such as immune checkpoint inhibition.[7] In contrast, non-mutated, naturally presented chronic lymphocytic leukaemia-associated antigens, which we previously identified using mass spectrometry-based immunopeptidome analysis,[8] elicit spontaneous immune responses that are associated with improved disease outcome in patients with chronic lymphocytic leukaemia.[9,10] Thus, therapeutic strategies to induce T cells that specifically target these chronic lymphocytic leukaemia-associated antigens might positively affect disease outcomes.[11]

iTAC-XS15-CLL is a personalised multipeptide-based T-cell activator, composed on the basis of HLA allotyping and immunopeptidome analysis of individual patients with chronic lymphocytic leukaemia. The workflow used a premanufactured peptide warehouse containing frequent chronic lymphocytic leukaemia-associated HLA class I-restricted and HLA class II-restricted peptides,[8] enabling fast-track personalised composition.[12] Selected peptides were adjuvanted with the Toll-like receptor (TLR) 1/2 agonist XS15[13] emulsified in Montanide ISA 51 VG to promote the activation and maturation of antigen-presenting cells and prevent peptide degradation. In the past few years, XS15-adjuvanted peptide-based T-cell activators have been proven to induce long-lasting T-cell responses, even in highly immunocompromised patients.[13–17]

Here, we report on an open-label, investigator-initiated, phase 1 trial evaluating iTAC-XS15-CLL01 in patients with chronic lymphocytic leukaemia who were undergoing BTKi treatment. We evaluated the immunogenicity of this agent by comparing the induction of T-cell responses after application of iTAC-XS15-CLL01 with the patients' baseline responses and monitored the frequency and severity of adverse events from the first application to the end of treatment.

Methods

Study design and participants

This single-arm, open-label, phase 1 trial was designed by and conducted at the Clinical Collaboration Unit Translational Immunology, University Hospital Tübingen (Tübingen, Germany). Patients were eligible for inclusion if they had a documented diagnosis of chronic lymphocytic leukaemia according to the International Workshop on chronic lymphocytic leukaemia (iwCLL) guidelines, were aged 18 years or older, and were due to receive a BTKi-based regime either as monotherapy or in combination (eg, with anti-CD20), according to the treating physician's choice and irrespective of treatment line. As standard treatment guidelines changed during the trial, choice of BTKi was left to the treating physician and not restricted to

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one particular drug. To accommodate this approach, the protocol was amended twice from its original solely ibrutinib-based form to include acalabrutinib and zanubrutinib; both amendments were approved by the regulatory authorities. Additionally, patients had to have an Eastern Cooperative Oncology Group score of 2 or lower as well as a negative serological hepatitis B test (or negative PCR test in the case of a positive serological test without evidence of an active infection), a negative test for hepatitis C RNA, and a negative HIV test (all within 6 weeks of inclusion in the study). The patients' HLA allotype had to match at least one of the corresponding HLA alleles of peptides included in the peptide warehouse (HLA-A*02, HLA-A*24, and HLA-B*07). To begin treatment with iTAC-XS15-CLL01, patients had to have attained at least partial remission with remaining MRD positivity after 6–8 months of BTKi therapy. Key exclusion criteria included treatment regimens without BTKi, disease transformation (ie, Richter's syndrome and prolymphocytic leukaemia), autoimmune haemolysis or immune thrombocytopenia caused by chronic lymphocytic leukaemia, pre-existing autoimmune disease, and any immunosuppressive treatment not related to chronic lymphocytic leukaemia, except corticosteroids. Because we were evaluating iTAC-XS15-CLL01 for the first time in humans, with no previous knowledge of its effects on pregnancy or its transmissibility via breast milk, pregnant and lactating patients were excluded, and patients had to agree to use contraceptive measures during the trial if they were still of reproductive potential. Full details of inclusion and exclusion criteria are included in the appendix (pp 2–3).

See **Online** for appendix

Patient feedback gained in our previous and ongoing trials with T-cell activators in other entities[15,17–19] was implemented in the study design, particularly regarding the number of study visits, diagnostic measures outside of standard guidelines (eg, frequency of bone marrow diagnostics or CT scans), and composition of the patient diary. All patients provided written informed consent before enrolment. The study was approved by the ethics committees of the University Hospital Tübingen (554/2020AMG) and the Paul Ehrlich Institute (4145). The trial was registered on ClinicalTrials.gov (NCT04688385) and EudraCT (number 2020-002367-65). The protocol has been published.[20] Safety oversight was provided by an independent data safety monitoring board.

Procedures

The health status of study patients was assessed by medical history and laboratory test values, assessment of vital signs, and physical examination at screening, before the start of BTKi treatment. Ethnicity, gender, and biological sex were self-reported. Laboratory tests to assess eligibility comprised haematology and clinical chemistry to establish chronic lymphocytic leukaemia status and organ function; HLA typing; serological testing for HIV, hepatitis B, and hepatitis C; immunophenotyping; and, in female patients, a pregnancy test.

Participants underwent a total of eight visits, consisting of the baseline screening visit, visits at 3–4 months and 6–8 months after BTKi initiation, three visits for application of iTAC-XS15-CLL01 (V1–V3), a visit 4–6 weeks after the last application (end of treatment), and a final follow-up visit 6 months (±14 days) after the last application (end of study). Interim treatment outcomes were assessed after 6–8 months of BTKi-based treatment, before study patients entered the T-cell activator application phase. On the basis of our previous work,[15,17] participants received three doses of iTAC-XS15-CLL01, injected subcutaneously into the lower part of the abdomen at monthly intervals (ie, three treatments across 2 months) while BTKi therapy was continued. No dose modification of the T-cell activator was included in the trial. However, the protocol specified that if T-cell activator-related adverse events of grade 3 or worse or any related skin ulceration occurred, application was to be interrupted and only resumed if the adverse event resolved to grade 2 or lower and any ulceration had completely resolved. Patients were to be prematurely removed from the trial if there was withdrawal of informed consent, a major protocol violation, progressive disease, a serious adverse event caused by the study drug, pregnancy in female patients, and if continuation of the trial would be detrimental to the subject's wellbeing.

Imaging of lymph nodes and spleen was done at the screening visit, 6–8 months after BTKi initiation, at the first and third application of T-cell activator, and at end of treatment (4–6 weeks after the last application). To keep radiation exposure to a minimum, CT scans were only done at the visit 6–8 months after BTKi initiation; at the other timepoints, imaging was sonographic. Haematology, laboratory, and clinical chemistry assessments were done at screening, 3–4 months after BTKi initiation, 6–8 months after BTKi initiation, every iTAC-XS15-CLL01 application, end of treatment, and end of study. Bone marrow aspirations with MRD evaluation were done by flow cytometry (level of resolution for MRD 1 in 10^4] 6–8 months after initiation of BTKi therapy and at end of treatment. In 11 samples, coagulation prevented evaluation by flow cytometry. However, to keep the extra burden for patients to a minimum, sampling was documented as not done rather than being repeated. MRD evaluations in peripheral blood were done at screening, 3–4 and 6–8 months after BTKi initiation, V3, end of treatment, and end of study.

Assessment of immune response was done using IFNγ-ELISPot assays at every visit for application of iTAC-XS15-CLL01 (with blood taken before injection of the T-cell activator), as well as at end of treatment and end of study. The assays were done after obtaining and processing the last blood sample of each patient at end of

study to limit inter-assay variability. To enable detection of low-frequency T-cell responses, peripheral blood mononuclear cells (PBMCs) were pulsed with HLA class I (1 µg/mL) or HLA-DR (5 µg/mL) peptide pools and cultured for 12 days with the addition of interleukin-2 (20 U/mL) on days 3, 5, and 7; they were then stimulated with 1 µg/mL of HLA class I-restricted peptides or 2·5 µg/mL of HLA class II-restricted peptides. Samples were analysed in technical triplicates when possible. Spots were counted using an ImmunoSpot S5 analyser (CTL; Shaker Heights, OH, USA). iTAC-XS15-CLL01-induced T cells were further characterised by flow cytometry-based cell surface marker and intracellular cytokine staining as well as by single-cell RNA sequencing for immune cell and T-cell receptor (TCR) profiling.

From the second application of iTAC-XS15-CLL01 (ie, V2), patients were assessed for adverse events at every visit until end of treatment, including an evaluation of patient diaries (covering 28 days after each application (ie, with the diary starting from V1), graded by study investigators according to a Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 grading scale (appendix p 10). Safety assessment included clinically significant changes in laboratory values (haematology and blood chemistry) reported as adverse events and serious adverse events (which included COVID-19). Adverse events were categorised and graded according to CTCAE version 5.0 and systematically assessed; injection-site events were graded with a modified CTCAE. Per protocol, the relationship of the application of iTAC-XS15-CLL01 to adverse events was assessed by the treating investigators (JSH, SJ, CH, HRS, and MM).

The chronic lymphocytic leukaemia peptide warehouse was developed and produced by the Peptide Laboratory at the Department of Peptide-based Immunotherapy, Institute of Immunology, University of Tübingen, Germany, according to Good Manufacturing Practices. The warehouse comprised nine HLA class I chronic lymphocytic leukaemia-associated peptides (three each for HLA-A*02, HLA-A*24, and HLA-B*07) with coverage of 87% in the previously published chronic lymphocytic leukaemia cohort,[8] three HLA class II chronic lymphocytic leukaemia-associated peptides with promiscuous binding to various different HLA class II allotypes, and two HLA class II control peptides (appendix p 9). Apart from the control peptides, these peptides are chronic lymphocytic leukaemia-associated T-cell epitopes frequently and exclusively presented in the immunopeptidome of patients with chronic lymphocytic leukaemia compared with a large benign tissue sample-derived immunopeptidome database.[8]

Liquid chromatography-coupled tandem mass spectrometry-based immunopeptidome analyses were conducted on individual study patients' PBMCs isolated at screening. The cells were lysed for subsequent immunoprecipitation of HLA-peptide complexes with monoclonal antibodies W6-32 (HLA class I) or L243 and Tü-39 (HLA class II; all produced in-house) and acidic elution of HLA-restricted peptides followed by analysis on an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific; Waltham, MA, USA). iTAC-XS15-CLL01 comprised three personalised HLA class I peptides, selected from the peptide warehouse on the basis of HLA allotyping and immunopeptidome analyses, supplemented in each case by all five available HLA class II-restricted peptides (three chronic lymphocytic leukaemia-specific and two control peptides). Selected peptides (300 µg per peptide) were adjuvanted with 50 µg of the synthetic lipopeptide TLR1/2 ligand XS15 (manufactured by Bachem; Bubendorf, Switzerland) and emulsified in Montanide ISA 51 VG (manufactured by Seppic; Paris, France).[13,15]

Outcomes

There were two primary endpoints. The first primary endpoint was frequency and severity of adverse events and serious adverse events associated with the application of iTAC-XS15-CLL01, assessed from the first application until end of treatment. The number and percentage of patients with adverse events until end of study were reported. The second primary endpoint was induction of a T-cell response after application of iTAC-XS15-CLL01, assessed at every visit from the first application onwards.

Secondary endpoints were duration of response, defined as time from best overall response (either complete response, complete response with incomplete blood count recovery, or partial response) until progression (not yet reached, therefore not reported here); MRD negativity rate in peripheral blood and bone marrow; and reduction rate of MRD in peripheral blood and bone marrow, calculated by comparing the flow cytometry results from consecutive visits. MRD assessment was done centrally at the Haematology Laboratory at the University Hospital Schleswig-Holstein, Campus Kiel (Kiel, Germany). Additional secondary objectives were immunophenotyping, including absolute changes in the number and percentage of lymphocyte subset counts between baseline (ie, at V1, before the first application of T-cell activator) and end of study; progression-free survival; and quality of life (European Organisation for Research and Treatment of Cancer [EORTC] quality of life questionnaire [QLQ] C-30). Progression-free survival and quality of life after end of study are not reported here.

As exploratory endpoints, we evaluated the correlation between inducibility of immune responses and clinical, biological (including post-hoc single-cell RNA sequencing), and immunopeptidomic patient characteristics, as assessed at baseline as well as before and after application of iTAC-XS15-CLL01. We also assessed overall survival, disease-free survival, and remission status at 2 years and 5 years after end of study. Due to the long-term nature of these analyses, they will be reported in a follow-up publication, as will the results of quality-of-life analyses,

which are still ongoing. Further details of the methods are provided in the appendix (pp 3–7, 16).

Statistical analysis

The sample size of 20 patients was calculated on the basis of the assumption that in the case of peptide-specific immune response induction in 30% or fewer of the patients (null hypothesis), the probability of incorrectly rejecting the null hypothesis would be 5% at most (type 1 error; one-sided). In the case of peptide-specific immune response induction in 60% or more of the patients (alternative hypothesis), the probability of correctly rejecting the null hypothesis would be 80% (ie, 80% power. The exact values for power and type 1 error were 87% and 4·8%, respectively (calculations based on binomial distribution with n=20, p=0·3 or p=0·6, k<10 or k≥10). With the sample size of 20 patients, an immune response in at least ten patients was required for the therapy concept to go on to be evaluated in a randomised phase 2 study.

The analyses included relative and absolute frequency of peptide-specific T-cell responses (including a two-sided 95% CI for proportions, calculated with the Clopper–Pearson method); MRD reduction rates (absolute and percentage changes from baseline [ie, 6–8 months after BTKi initiation] to end of treatment to end of study; assessments were done in blood and bone marrow but only results in blood are reported); MRD negativity rate (defined as blood or bone marrow with fewer than one chronic lymphocytic leukaemia cell per 10 000 leukocytes according to iwCLL criteria[21]); overall survival; progression-free survival; absolute and percentage changes in immunophenotype, blood count, and immunoglobulin parameters for the respective visits in comparison with baselines; and safety.

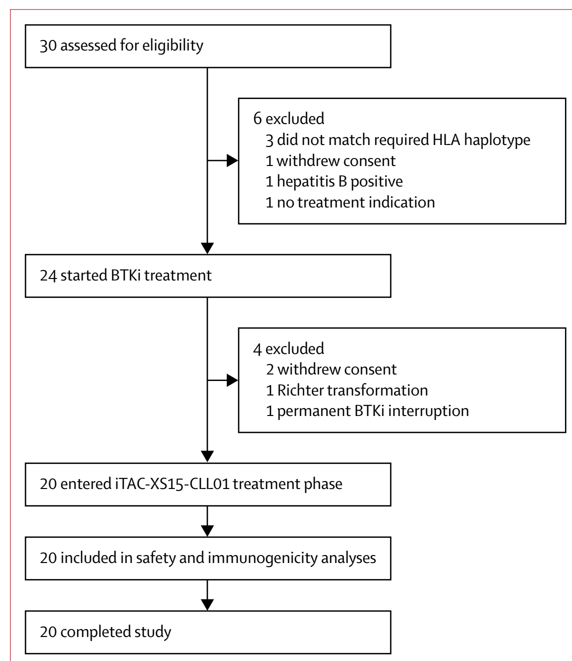

Figure 1: **Trial profile**
BTKi=Bruton's tyrosine kinase inhibitor.

For adverse events that occurred only once in a patient, safety data were summarised by counting each adverse event using the lowest level term. If the same adverse event occurred more than once in the same patient, only the highest-graded adverse event was counted. The predefined stopping rule compromised the occurrence of more than three drug-related serious adverse events. Results are reported as frequencies, means with SDs, medians and IQRs (defined as first and third quartiles), box plots showing medians with 25% or 75% quantiles and minimum–maximum whiskers, and rates with 95% CIs. For exploratory (ad-hoc) analysis (association of T-cell responses with baseline values of the different compartments of the immune system, BTKi treatment, treatment-related adverse events, and MRD reduction), we generated correlation coefficients (*r*) and p values with Spearman's test. In addition, linear regression analysis was done, yielding R^2 and p values. For the comparison of different assessment timepoints, we conducted the Friedman test followed by the Dunn–Bonferroni test for multiple comparison to V1. For post-hoc data analysis of single-cell RNA sequencing, the neighbourhood graph and Uniform Manifold Approximation and Projection (UMAP) embedding were computed using the UMAP algorithm, and unsupervised clustering was done with the Leiden algorithm. CD3 clusters were selected for subclustering with the same algorithms. Differentially expressed genes were calculated using the Wilcoxon rank-sum test with Benjamini–Hochberg adjustment and tie correction. Cellranger output tables were demultiplexed and analysed per patient, identifying clonotypes on the basis of their CDR1, CDR2, and CDR3 amino acid sequences and V(D)JC gene alignments. Clonotypes expanded (≥5 clones) within the patient PBMC samples obtained at end of treatment (and cultured as described) were normalised to 10 000 cells, tracked across samples, and assessed for significant expansion using $\log_2$ fold change greater than 1 and a one-sided Fisher's exact test with Bonferroni-adjusted p value lower than 0·05. Flow cytometric data were analysed using FlowJo version 10.10.0 (BD Biosciences; Franklin Lakes, NJ, USA). Graphs were plotted using Rstudio and GraphPad Prism 10. Statistical analyses were conducted using GraphPad Prism 10 and SAS version 9.4.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

From Jan 21, 2021 to July 7, 2023, 30 patients were screened. Six patients were excluded: three did not match the required HLA class I allotypes, one withdrew consent

before entering the BTKi treatment phase, one was hepatitis B positive, and one had no treatment indication according to iwCLL guidelines. Of the 24 patients entering the BTKi treatment phase, one had Richter transformation during BTKi treatment, one had extensive interruption of BTKi therapy due to a SARS-CoV-2 infection, and two withdrew consent. Thus, 20 patients entered the iTAC-XS15-CLL01 treatment phase (figure 1; appendix pp 17–18). All patients were diagnosed with chronic lymphocytic leukaemia according to iwCLL guidelines, three (15%) with Binet stage A, 13 (65%) with Binet stage B, and four (20%) with Binet C disease (table 1). Patient age ranged from 37·0 years to 78·0 years (median 56·5 years [IQR 49·5–65·5]). Six (30%) patients were female, and 14 (70%) were male; all were of White ethnicity. According to chronic lymphocytic leukaemia international prognostic index scores, nine (45%) patients were classified as being at intermediate risk, eight (40%) as being at high risk, and three (15%) as being at very high risk. Nine (45%) patients received BTKi treatment with ibrutinib, and 11 (55%) were treated with acalabrutinib, including one patient who received both ibrutinib and acalabrutinib sequentially; one (5%) received zanubrutinib. All patients completed the trial, including the end of study visit; one patient discontinued BTKi treatment after the end of treatment visit. All patients had attained a partial response under BTKi treatment before the first application of iTAC-XS15-CLL01, and no patient had reached a complete response.

Warehouse-based design and production of the personalised T-cell activator was feasible for all study patients (appendix p 20). Ten (50%) study patients were positive for HLA-A*02, seven (35%) for HLA-A*24, and seven (35%) for HLA-B*07; four (20%) were positive for two allotypes (appendix pp 17–18). Immunopeptidome analysis to guide personalised peptide selection was feasible in 16 (80%) patients with sufficient amounts of PBMCs available for HLA ligand isolation (appendix pp 17–18). We identified 3787–19 310 (median 10 339 [IQR 6287–16 817]) HLA class I ligands and 1841–18 442 (median 11 668 [5404–14 909]) HLA class II-restricted peptides (appendix p 24). Total peptide yields did not correlate with PBMC counts. At least two warehouse peptides (median four) were identified in the immunopeptidomes of each of the 16 patients (appendix pp 17–18, 20). Peptide detection rates ranged between 19% (three of 16 patients) and 100% in the patients' immunopeptidomes (median 83% [IQR 50–100]; appendix pp 17–18). Detection of allotype-normalised warehouse peptides correlated with PBMC counts for all peptides. Identification of HLA-normalised warehouse peptides correlated with overall peptide yields for HLA class I-restricted peptides (appendix p 24).

Safety data were available for all patients from safety visits from VI until end of study. Local treatment-related adverse events—ie, expected reactogenicity at the

	Total (N=20)
Age, years*	56·5 (49·5–65·5)
Sex†	
Female	6 (30%)
Male	14 (70%)
Ethnicity	
White	20 (100%)
Rai stage	
I	6 (30%)
II	11 (55%)
III	1 (5%)
IV	2 (10%)
Binet stage	
A	3 (15%)
B	13 (65%)
C	4 (20%)
IGHV status	
Unmutated	16 (80%)
Mutated	3 (15%)
TP53 status	
Unmutated	18 (90%)
Mutated	2 (10%)
CLL-international prognostic index score	
0–1	0 (0%)
2–3	9 (45%)
4–6	8 (40%)
7–10	3 (15%)
BTKi treatment‡	
Ibrutinib	9 (45%)
Acalabrutinib	11 (55%)
Zanubrutinib	1 (5%)
Disease stage §	
Partial remission	20 (100%)
Complete remission	0
Remaining CLL population before application of iTAC-XS15-CLL0A1¶	
CLL cell population, peripheral blood	44·6% (17·4–84·8)
CLL cell population, bone marrow	43·9% (26·5–82·3)
Lymphocyte count, cells per µL	6795 (4200–48 605)
Immune status, cells per µL	
CD4-positive	1048 (774·5–1495·0)
CD8-positive	680·5 (424·5–1095·5)
Applications of iTAC-XS15-CLL01 received	
First	20 (100%)
Second	20 (100%)
Third	17 (85%)

Data are n (%) or median (IQR) unless otherwise specified. Data were assessed at the time of screening except where otherwise indicated. BTKi=Bruton's tyrosine kinase inhibitor. CLL=chronic lymphocytic leukaemia. *Range 37·0–78·0 years. †Self-reported gender matched self-reported sex in all participants. ‡One patient switched from ibrutinib to acalabrutinib. §Assessed at visit 6–8 months after BTKi initiation. ¶Assessed after the last patient had reached end of study.

Table 1: **Patient characteristics**

	Grade 1–2	Grade 3
Cardiac disorders		
Atrial flutter*	0	1 (5%)
Gastrointestinal disorders		
Dental caries	0	1 (5%)
General disorders and administration site conditions		
Influenza-like symptoms	9 (45%)	0
Fever	2 (10%)	0
Vaccination-site lymphadenopathy	2 (10%)	0
Infections and infestations		
COVID-19*	3 (15%)	0
Lung infection	0	1 (5%)
Borreliosis	1 (5%)	0
Injury, poisoning, and procedural complications		
Vaccination-site complication		
Erythema	17 (85%)	3 (15%)
Granuloma	18 (90%)	2 (10%)
Pain	11 (55%)	0
Swelling	15 (75%)	0
Ulceration	6 (30%)	1 (5%)
Musculoskeletal and connective tissue disorders		
Back pain*	0	1 (5%)
Nervous system disorders		
Headache	2 (10%)	0
Vascular disorders		
Hypertension	2 (10%)	0

Data are n (%) for all patients (N=20). Only grade 1–2 adverse events occurring in 10% or more of participants are reported, and no grade 4 or 5 adverse events were reported. CTCAE=Common Terminology Criteria for Adverse Events. *Serious adverse events; as per the protocol, every SARS-CoV-2 infection was reported as a serious adverse event.

Table 2: **Summary of all treatment-emergent adverse events, by system organ class and CTCAE term**

application site—occurred in all patients (table 2; appendix pp 17–18). No treatment-related serious adverse events and no grade 4 adverse events were reported. Application-site adverse events were mild to moderate (grade 1–2) in 14 (70%) patients. All patients showed the expected formation of a granuloma at the injection site. Other injection-site adverse events included erythema in all patients, swelling in 15 (75%), pain in 11 (55%), skin ulceration in seven (35%), and lymphadenopathy in two (10%), with an increase in the number and severity observed with successive applications. Grade 3 injection-site adverse events occurred rarely and comprised local erythema in three (15%) patients, granuloma in two (10%), and skin ulceration (>2 cm, with no substantial damage or necrosis of subcutaneous tissue) in one (5%; appendix pp 17–19). One patient received topical treatment for the skin ulceration, resulting in resolution; in all other cases, ulceration healed without intervention. In three (15%) patients, local injection-site reactions after two applications of iTAC-XS15-CLL01 led to the third application not being done. Systemic application-related adverse events occurred in four (20%) patients and were mostly mild (ten [77%] of 13 events]), not exceeding grade 2. The most frequently reported systemic-related adverse events were influenza-like symptoms (two [10%] patients) and fever (two [10%] patients); the cases of fever occurred after the second or third application (appendix pp 17–18). Overall, 128 adverse events were reported for all patients, of which 46 (36%) were judged to be not related to the treatment. Apart from application-site reactions, the most frequently observed treatment-emergent adverse events were influenza-like symptoms in nine (45%) patients and COVID-19 in three (15%). After the first application, five serious non-treatment related adverse events were reported for four (20%) patients: one case of atrial flutter (grade 3), one case of back pain (grade 3), and three cases of COVID-19 (grade 1; appendix pp 17–18). With regard to infectious disease-related adverse events in particular, there occurred one case of urinary tract infection (grade 2), one case of pneumonia (grade 3), four cases of COVID-19 (grade 1), and one case of herpes simplex reactivation (grade 2; appendix pp 17–18). No death, treatment-related or otherwise, occurred during the trial, and no patient discontinued the trial for drug-related toxicity. Although influenza-like symptoms (grade 1) and, in two cases, fever (grade 2) occurring immediately after injection-site reactions constituted immune-mediated conditions, we did not observe cytokine release syndrome higher than grade 1, immune effector cell-associated neurotoxicity syndrome, or any long-term immune-mediated medical condition up to the last study visit in any patient.

iTAC-XS15-CLL01-induced peptide-specific IFN-γ T-cell responses were documented in 19 (95% [95% CI 75·1–99·9]) study patients at end of treatment (figure 2A), increasing by more than 100-fold from baseline (median calculated spot counts 4·0 [IQR 2·0–23·5] at V1 [before injection of iTAC-XS15-CLL01] and 445·0 [97·2–2115·0] at end of treatment). There was higher induction of T-cell responses to chronic lymphocytic leukaemia-associated HLA class-II restricted peptides from baseline to end of treatment than to HLA class-I restricted peptides (figure 2A, B; appendix pp 20–21). Four (20%) patients showed low-frequency pre-existing T-cell responses to at least one applied peptide (responses to HLA class I peptides in three patients and to HLA class II peptides in one patient; figure 2A). iTAC-XS15-CLL01 induced T-cell responses against multiple peptides (median four [IQR two to four]; n=17 patients). T-cell responses persisted until the end of study visit in 16 (84%) of 19 patients, increasing further in intensity (figure 2A). The number of patients with iTAC-XS15-CLL01-induced T-cell responses to peptides of both HLA class I and class II increased from seven (35%) of 20 at end of treatment to eight (42%) of 19 at end of study (figure 2B). There were pre-existing T-cell responses against HLA class II-restricted control peptides in eight (40%) of 20 patients, increasing with

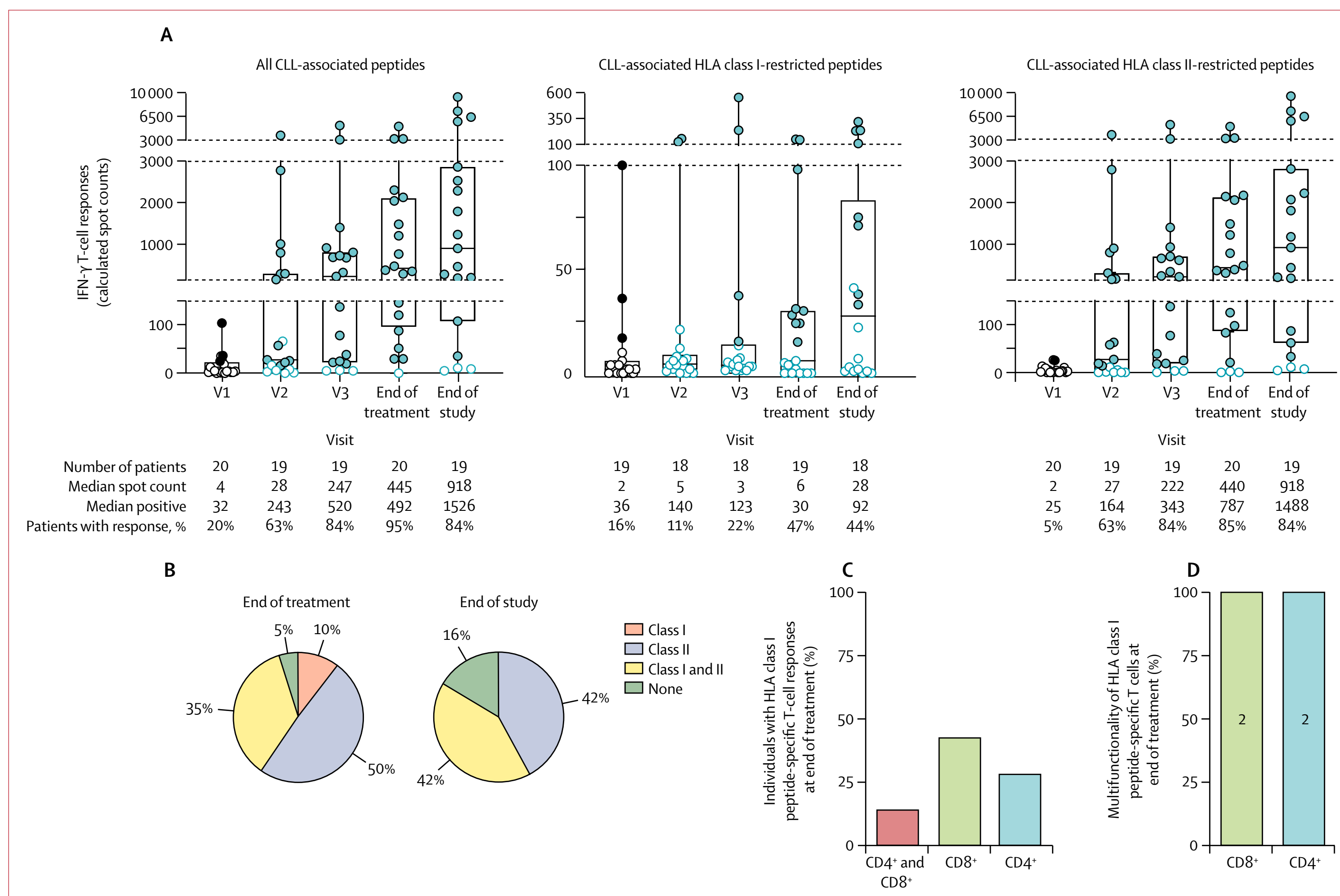

***Figure 2:* iTAC-XS15-CLL01-induced T-cell response**

(A) iTAC-XS15-CLL01-specific T-cell responses at baseline (V1, before injection of iTAC-XS15-CLL01) and different timepoints after application (1 month after the first application [V2], 1 month after the second application [V3], one month after the third application [end of treatment], and 6 months after the last application [end of study]). Pre-existing T-cell responses are depicted in black, filled dots represent responders, and empty dots represent non-responders. Box plots indicate the 25% and 75% quantiles; whiskers show minimum and maximum values, and the horizontal line in each box indicates the median value. Median positive refers to the median IFN-γ T-cell response in patients assessed as having an iTAC-XS15-CLL01-induced immune response. (B) Proportion of donors with T-cell responses targeting associated T-cell activator peptides restricted for HLA class I, HLA class II, HLA class 1 and II, or neither at end of treatment and end of study. (C, D) Characterisation of iTAC-XS15-CLL01-induced T-cell responses using intracellular cytokine and cell surface marker staining. Frequencies of CLL-associated HLA class I-restricted T-cell responses (CD4-positive, CD8-positive or both; C) and multifunctionality of HLA class I-restricted T cells (D) at end of treatment (numbers within the columns are the numbers of activation markers detected).

iTAC-XS15-CLL01 application to 17 (85%) of 20 by end of treatment and 17 (89%) of 19 by end of study (appendix pp 21–22). iTAC-XS15-CLL01-induced chronic lymphocytic leukaemia-specific T-cell responses were mediated by polyfunctional CD4-positive and CD8-positive T cells (figure 2C, D; appendix pp 21–22). At end of treatment, responses targeting HLA class I-derived chronic lymphocytic leukaemia-associated peptides were mediated by CD8-positive T cells in three (43%) of seven patients with intracellular staining data available, by CD4-positive T cells in two (29%) patients, and by both CD4-positive and CD8-positive T cells in one (14%) patient; the T cells had positivity for CD107a, IFNγ, or TNF (or any combination thereof; figure 2C; appendix pp 21–22). Of 14 patients with intracellular staining data available, iTAC-XS15-CLL01-induced T-cell responses targeting HLA class II-derived chronic lymphocytic leukaemia-associated peptides at end of treatment were mediated by CD4-positive T cells in 11 (79%), by CD8-positive T cells in four (29%), and both CD4-positive and CD8-positive T cells in three (21%; appendix pp 21–22). All CD4-positive and most CD8-positive (three [75%] of four) T-cell responses were polyfunctional, with increased cell surface marker expression or cytokine release (CD107a, IFNγ, or TNF or any combination thereof; appendix pp 21–22). T-cell responses remained polyfunctional until end of study, with responses mediated by CD4-positive and CD8-positive T cells for HLA class I-restricted peptides (CD4-positive T cells in three [43%] of seven patients, CD8-positive T cells in one [14%], and CD4-positive and CD8-positive T cells in one [14%]) as well as for HLA class II-restricted peptides (CD4-positive T cells in 13 [81%] of 16 patients, CD8-positive T cells in nine [56%], and CD4-positive and CD8-positive T cells in four [24%];

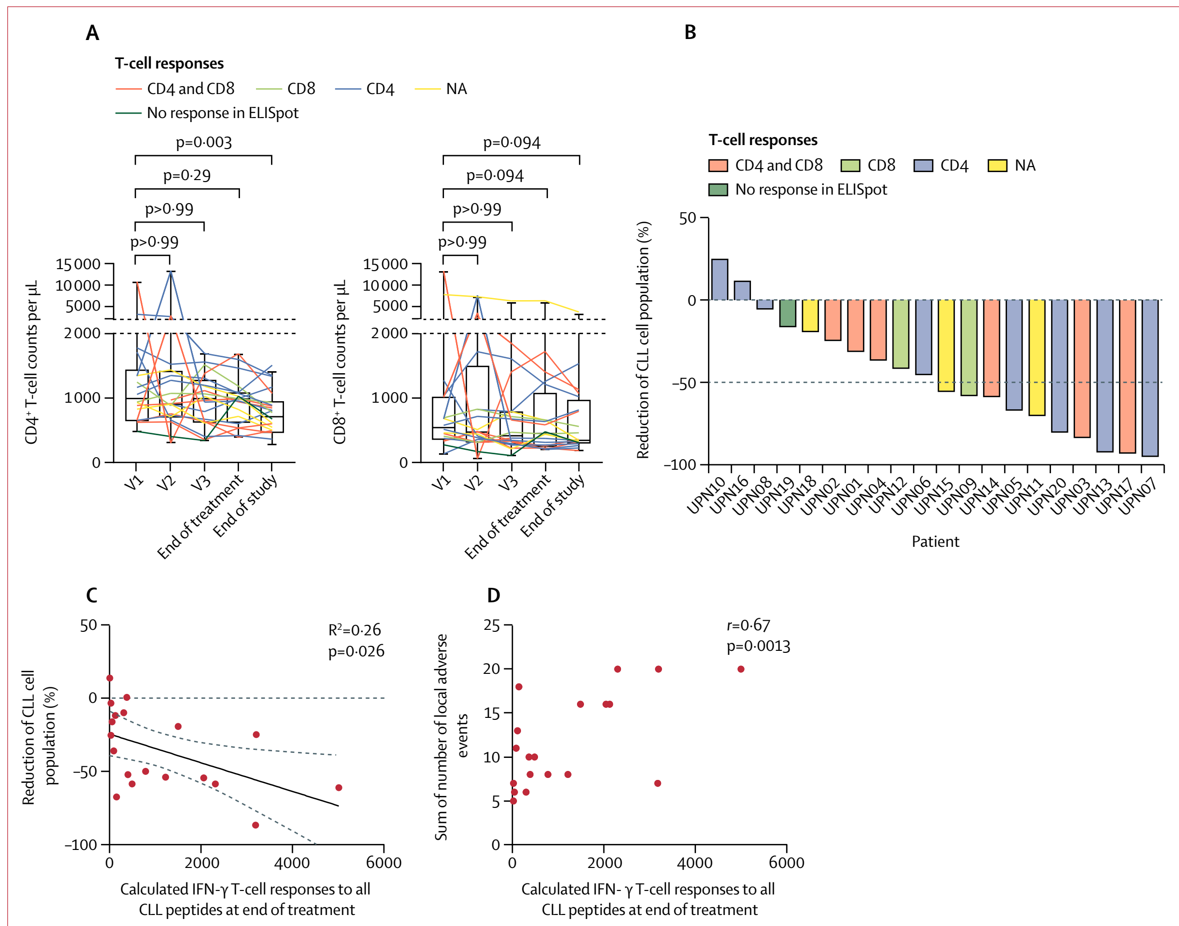

Figure 3: **iTAC-XS15-CLL01-induced immune response correlates with clinical response**
(A) Changes in CD4-positive and CD8-positive T-cell counts during treatment with iTAC-XS15-CLL01. Line plots are shown for individual patients, with the type of T-cell responses induced indicated by colour. Box plots indicate 25% and 75% quantiles; whiskers show minimum and maximum values, and the horizontal line in each box indicates the median value. V1–V3 are the patient visits for application of iTAC-XS15-CLL01. (B) Waterfall plot showing activity of iTAC-XS15-CLL01 in terms of reduction of the proportion of chronic lymphocytic leukaemia cells at end of study and T-cell responses against chronic lymphocytic leukaemia-associated T-cell activator peptides until end of study; type of T-cell response induced in each patient is indicated by colour. (C) Linear regression analysis showing correlation between T-cell responses against chronic lymphocytic leukaemia-associated T-cell activator peptides and reduction of the proportion of chronic lymphocytic leukaemia cells at end of treatment; solid and dashed lines represent the linear regression and 95% CI, respectively. (D) Correlation (Spearman) between T-cell responses against chronic lymphocytic leukaemia-associated T-cell activator peptides (until end of treatment) and number of local adverse events (until end of study) per patient. CLL=chronic lymphocytic leukaemia. NA=not available.

appendix pp 21–22). All three chronic lymphocytic leukaemia-associated HLA class II-restricted peptides contain embedded sequences predicted as binders for several of the HLA allotypes of study patients showing CD8-positive T-cell responses to an HLA class II peptide (appendix p 23).

Median absolute CD4-positive and CD8-positive T-cell counts showed no relevant variations during the treatment phase until end of study and, in exploratory analyses, there were no relevant associations between absolute cell counts and patients' individual iTAC-XS15-CLL01-induced CD4-positive or CD8-positive T-cell responses, or both (figure 3A). iTAC-XS15-CLL01-induced chronic lymphocytic leukaemia-specific T-cell responses correlated negatively with white blood cell, lymphocyte, and B-cell counts assessed at V1 (before the first application of iTAC-XS15-CLL01; appendix pp 12, 28–29). No other baseline value of the different compartments of the immune system (including absolute numbers per mL of CD4-positive and CD8-positive T cells) was associated with activator-induced T-cell responses.

At end of study, all study patients were alive (appendix p 13), with no progression of chronic lymphocytic leukaemia observed. Reduction of the chronic lymphocytic leukaemia cell population compared with baseline (ie 6–8 months after initiation of BTKi) was observed in 18 (90·0% [95% CI 68·3–98·8]) of 20 patients (figure 3B; appendix pp 14, 15, 28–30), with a median decrease of 50·0% (IQR 74·9–21·9) compared with baseline. In exploratory analyses, the highest reduction rates were observed in patients with iTAC-XS15-CLL01-induced CD4-positive T-cell responses

or both CD4-positive and CD8-positive T-cell responses. However, two patients showed CD4-positive responses without an accompanying reduction in chronic lymphocyctic leukaemia burden. Overall reduction of chronic lymphocytic leukaemia cells correlated with iTAC-XS15-CLL01-induced T-cell responses at end of treatment and showed a linear association ($R^2=0·2596$; $p=0·026$; figure 3C). A direct association between increased iTAC-XS15-CLL01-induced T-cell responses and a decrease in the chronic lymphocytic leukaemia cell population, as well as decreasing lymph node size, is shown for individual patients in the appendix (pp 28–29).

Exploratory analyses showed that iTAC-XS15-CLL01-induced T-cell responses were of lower intensity in patients receiving acalabrutinib than in those receiving ibrutinib (appendix p 28). Regarding treatment-related adverse events and immunogenicity, application-site reactions were positively correlated with iTAC-XS15-CLL01-induced T-cell responses (figure 3D; appendix pp 12, 28). Of note, at any assessment after the last application of iTAC-XS15-CLL01, the median intensity of T-cell response in patients who could not receive a third application did not differ significantly from that of patients who received three applications (appendix pp 28–29).

In post-hoc analyses, iTAC-XS15-CLL01-induced T-cell responses at end of treatment were characterised by single-cell RNA sequencing and single-cell TCR V(D)J sequencing of magnetically sorted and peptide-stimulated T cells from two patients. iTAC-XS15-CLL01-induced clonal expansion was observed, with nine substantially expanded T-cell clones for patient UPN02 and four for patient UPN06, matching the iTAC-XS15-CLL01-induced T-cell responses observed in the IFNγ ELISpot assay (appendix pp 25–27). By using the TCRdist nearest neighbour algorithm (appendix pp 5–7), the set of potential T-cell activator-induced TCR clones was expanded, with up to 13 nearest neighbours (median two [range none to 13; IQR none to two]) with high TCR sequence similarity identified for the iTAC-XS15-CLL01-induced clones (appendix pp 11, 25). Unsupervised clustering of single-cell RNA sequencing data identified seven main T-cell clusters (CD4-positive naive, CD4-positive memory, CD4-positive effector memory, CD4-positive T helper 2 [Th2], CD8-positive naive, CD8-positive memory, and CD8-positive cytotoxic), with iTAC-XS15-CLL01-expanded clones primarily assigned to the memory or cytotoxic CD8-positive T-cell cluster and, to a lesser extent, the CD4-positive Th2 T-cell cluster (appendix p 25).

Discussion

In this study, we showed that in patients with chronic lymphocytic leukaemia who were undergoing BTKi treatment, iTAC-CLL-XS15, a warehouse-based, personalised, multipeptide T-cell activator consisting of chronic lymphocytic leukaemia-associated antigens was safe and induced potent T-cell responses that lasted at least 6 months and were associated with decreases in the proportion of chronic lymphocytic leukaemia cells.

Therapeutic approaches inducing specific T-cell responses against cancer have emerged in the past few years.[22–24] Initial results promise the induction of long-term immune memory and sustained cancer control. However, the broad applicability of neoepitopes as therapeutic targets is restricted by patient individuality, tumour specificity, intratumoural heterogeneity of somatic mutations,[25] as well as the small number of mutations actually presented as HLA-restricted neoepitopes. In contrast, iTAC-CLL-XS15 is composed of frequent chronic lymphocytic leukaemia-associated T-cell epitopes identified by mass spectrometry-based immunopeptidome analysis.[8,9] To date, immunopeptidomics has been the only unbiased high-throughput method to identify naturally presented HLA peptides on the cell surface of tumour cells, thus representing an invaluable tool for antigen selection in immunotherapy.[26]

iTAC-XS15-CLL induced a potent T-cell response in 95% of patients, exceeding immune responses from previously reported peptide-based or mRNA-based approaches in both frequency and intensity.[12,22] T-cell responses targeted multiple peptides, preventing possible immune escape due to single antigen loss.[27] iTAC-XS15-CLL01-induced T-cell response was mediated by multifunctional Th1 type CD4-positive T cells and CD8-positive T cells, which has been proven to be the optimal combination for effective antitumour activity.[8,28] Chronic lymphocytic leukaemia-specific T-cell responses increased until the 6 month follow-up, underscoring the potential of XS15-adjuvanted peptide-based T-cell activators to induce responses with only a few applications, in contrast to other approaches requiring multiple boosters.[22–24]

In line with previous findings for XS15-adjuvanted T-cell activators,[15,16] local injection-site adverse events correlated positively with iTAC-XS15-CLL01-induced T-cell responses. This finding points to local application-site reactions serving as a potential biomarker to predict response and facilitate optimisation of the number of applications needed. Furthermore, a high lymphocyte count at baseline before the first application was associated with reduced iTAC-XS15-CLL01-induced T-cell responses, which underscores the currently followed paradigm to use therapeutic T-cell activating methods in an adjuvant setting or MRD context to benefit from optimal effector T-cell-to-tumour-cell ratios.[18,22,24] The iTAC-XS15-CLL01-induced T-cell response did not correlate with chronic lymphocytic leukaemia-international prognostic index score or T-cell counts, supporting previous data showing that XS15-adjuvanted T-cell activators induce potent T-cell responses even in immunocompromised patients at high risk.[17]

Local granuloma formation was observed in all study patients, which was an expected and intended local reaction after administration of this XS15 and Montanide-based T-cell activator.[14–17] Granuloma formation enables

continuous local stimulation of antigen-specific T cells required for induction of long-lasting T-cell responses without systemic inflammation. The low level of side-effects observed with our T-cell activation approach stands in stark contrast to other trials aiming at MRD elimination using combinatorial administration of approved drugs for treatment of chronic lymphocytic leukaemia, including antibody-based immunotherapy, chemotherapy, and small molecules, in various combinations and application regimes.[4] Although yielding substantial response and remission rates potentially higher than those seen in our trial, these approaches also resulted in drug toxicities, cytopenias, and infections.[3] Infections, drug toxicities, and cytopenias prevent older and comorbid patients with chronic lymphocytic leukaemia from taking full advantage of available treatment options, whereas our trial showed a very favourable benefit–risk ratio for iTAC-XS15-CLL01.

Activity analyses revealed a reduction of the proportion of chronic lymphocytic leukaemia cells in 90% of patients, with a median reduction of about 50%. MRD negativity was not detected in any of the study patients. However, there was only 6 months of observation time, which should be extended in follow-up trials. Of note, iTAC-XS15-CLL01-induced T-cell responses correlated with reduction of the proportion of chronic lymphocytic leukaemia cells in the study patients. Although randomised trials are required to clearly dissect the effect of iTAC-XS15-CLL01 from the backbone BTKi treatment, our data provide promising initial evidence for the positive effect of T-cell activator-induced T cells on clinical outcome.

Response assessment in the trial was limited by the sponsor's decision to keep radiation exposure of patients to a minimum. This stipulation meant that response assessment according to iwCLL criteria was not possible, as only one CT scan per patient was done. The small patient number, including only White and predominantly male patients, was a further limitation, as it cannot be clearly established how the results apply to larger populations, especially cohorts from other ethnic backgrounds with more widely spread HLA type compositions.

This single-arm first-in-human trial was designed to prove safety and tolerability of iTAC-XS15-CLLO01, as well as immunogenicity, in patients with chronic lymphocytic leukaemia under BTKi backbone regimens. The absence of a single-agent arm or placebo arm means that only preliminary data on the clinical activity of the T-cell activator were provided. Nonetheless, the safety and immunogenicity data established by this trial show that iTAC-XS15-CLL01 is a promising therapeutic T-cell activator that warrants further evaluation, which will be addressed in an upcoming randomised trial.

Contributors

HRS, H-GR, JSH, SW, and JSW were involved in the design of the study and strategy. YM, AN, and AD conducted the immunogenicity analyses. MW, NH-G, MLD, and JB conducted the immunopeptidome analyses. JSH, SJ, and JSW collected patient data and samples and conducted medical evaluation and analysis. JSH, CH, MM, SJ, and HRS collected data as study investigators. MoB conducted minimal residual disease assessment. PM developed the statistical design and oversaw the data analysis. A-SB, AL, CKr, GA, NK, MeB, and MZ conducted single-cell RNA sequencing analysis. MLD and MaP provided bioinformatic support for peptide selection. MD, LZ, CKa, MR, and MTO conducted Good Manufacturing Practice production of iTAC-XS15-CLL01. MPu and JSH conducted statistical analysis. JSH, SJ, YM, and JSW drafted the manuscript. JSH, YM, SJ, and JSW directly accessed and verified the underlying data reported in the manuscript. All authors contributed to review and editing of the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

H-GR, AN, and JSW are listed as inventors on patents related to the CLL vaccine peptides. H-GR is listed as inventor on a patent related to the adjuvant XS15. H-GR and JSW are shareholders of the spin-off company ViferaXS. H-GR, JSH, YM, MW, HRS, and JSW are listed as inventors on a vaccine patent that resulted from this trial. MoB declares research support and consulting fees by Amgen; payment for speakers bureaus from Amgen, Becton Dickinson, Jansson and Pfizer; travel support from Janssen; and participation in advisory boards of Incyte and Amgen, all of which are unconnected with the present study. All other authors declare no competing interests.

Data sharing

Data supporting the findings of this study, including de-identified patient data, are available after final completion of the trial report and will be shared according to data sharing guidelines on reasonable request to the corresponding author JSW (juliane.walz@med.uni-tuebingen.de). Data will only be shared for non-commercial interests and after ethical approval. A data use agreement is obligatory. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE[29] partner repository with the dataset identifier PXD059004; the repository can be accessed with the project accession number PXD059004 and token HoBPyhkKcXcN or with the username reviewer_pxd059004@ebi.ac.uk and password rq37Pcke65fe.The single-cell RNA sequencing data have been deposited to GEO (https://www.ncbi.nlm.nih.gov/gds) with the dataset identifier GSE305113; the access token is snixyyoeptsxxgf.

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References

1 Ou Y, Long Y, Ji L, et al. Trends in disease burden of chronic lymphocytic leukemia at the global, regional, and national levels from 1990 to 2019, and projections until 2030: a population-based epidemiologic study. *Front Oncol* 2022; **12:** 840616.

2 Coombs CC. Frontline therapy of CLL-changing treatment paradigms. *Curr Hematol Malig Rep* 2024; **19:** 65–74.

3 Patel K, Pagel JM. Current and future treatment strategies in chronic lymphocytic leukemia. *J Hematol Oncol* 2021; **14:** 69.

4 Seymour JF, Kipps TJ, Eichhorst BF, et al. Enduring undetectable MRD and updated outcomes in relapsed/refractory CLL after fixed-duration venetoclax-rituximab. *Blood* 2022; **140:** 839–50.

5 Schwarzbich MA, McClanahan F, Gribben J. Allogeneic transplantation for chronic lymphocytic leukemia in the age of novel treatment strategies. *Oncology* 2016; **30:** 526–33.

6 Riches JC, Davies JK, McClanahan F, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. *Blood* 2013; **121:** 1612–21.

7 Hallek M. Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. *Am J Hematol* 2019; **94:** 1266–87.

8 Nelde A, Maringer Y, Bilich T, et al. Immunopeptidomics-guided warehouse design for peptide-based immunotherapy in chronic lymphocytic leukemia. *Front Immunol* 2021; **12:** 705974.

9 Kowalewski DJ, Schuster H, Backert L, et al. HLA ligandome analysis identifies the underlying specificities of spontaneous antileukemia immune responses in chronic lymphocytic leukemia (CLL). *Proc Natl Acad Sci USA* 2015; **112:** E166–75.

10 Kowalewski DJ, Stevanovic S, Rammensee HG, Stickel JS. Antileukemia T-cell responses in CLL—we don't need no aberration. *OncoImmunology* 2015; **4:** e1011527.

11 Marconato M, Maringer Y, Walz JS, Nelde A, Heitmann JS. Immunopeptidome diversity in chronic lymphocytic leukemia identifies patients with favorable disease outcome. *Cancers* 2022; **14:** 4659.

12 Heitmann JS, Jung S, Wacker M, et al. Warehouse-based, immunopeptidome-guided design of personalised peptide vaccines shows feasibility in clinical trial evaluation in CLL patients. *Front Immunol* 2024; **15:** 1482715.

13 Rammensee HG, Wiesmüller KH, Chandran PA, et al. A new synthetic toll-like receptor 1/2 ligand is an efficient adjuvant for peptide vaccination in a human volunteer. *J Immunother Cancer* 2019; **7:** 307.

14 Bauer J, Köhler N, Maringer Y, et al. The oncogenic fusion protein DNAJB1-PRKACA can be specifically targeted by peptide-based immunotherapy in fibrolamellar hepatocellular carcinoma. *Nat Commun* 2022; **13:** 6401.

15 Heitmann JS, Bilich T, Tandler C, et al. A COVID-19 peptide vaccine for the induction of SARS-CoV-2 T cell immunity. *Nature* 2022; **601:** 617–22.

16 Tandler C, Heitmann JS, Michel TM, et al. Long-term efficacy of the peptide-based COVID-19 T cell activator CoVac-1 in healthy adults. *Int J Infect Dis* 2024; **139:** 69–77.

17 Heitmann JS, Tandler C, Marconato M, et al. Phase I/II trial of a peptide-based COVID-19 T-cell activator in patients with B-cell deficiency. *Nat Commun* 2023; **14:** 5032.

18 Jung S, Nelde A, Maringer Y, et al. AML-VAC-XS15-01: protocol of a first-in-human clinical trial to evaluate the safety, tolerability and preliminary efficacy of a multi-peptide vaccine based on leukemia stem cell antigens in acute myeloid leukemia patients. *Front Oncol* 2024; **14:** 1458449.

19 Hackenbruch C, Bauer J, Heitmann JS, et al. FusionVAC22_01: a phase I clinical trial evaluating a DNAJB1-PRKACA fusion transcript-based peptide vaccine combined with immune checkpoint inhibition for fibrolamellar hepatocellular carcinoma and other tumor entities carrying the oncogenic driver fusion. *Front Oncol* 2024; **14:** 1367450.

20 Englisch A, Hayn C, Jung S, et al. iVAC-XS15-CLL01: personalized multi-peptide vaccination in combination with the TLR1/2 ligand XS15 in CLL patients undergoing BTK-inhibitor-based regimens. *Front Oncol* 2024; **14:** 1441625.

21 Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood* 2018; **131:** 2745–60.

22 Platten M, Bunse L, Wick A, et al. A vaccine targeting mutant IDH1 in newly diagnosed glioma. *Nature* 2021; **592:** 463–68.

23 Latzer P, Zelba H, Battke F, et al. A real-world observation of patients with glioblastoma treated with a personalized peptide vaccine. *Nat Commun* 2024; **15:** 6870.

24 Rojas LA, Sethna Z, Soares KC, et al. Personalized RNA neoantigen vaccines stimulate T cells in pancreatic cancer. *Nature* 2023; **618:** 144–50.

25 Syga S, Jain HP, Krellner M, Hatzikirou H, Deutsch A. Evolution of phenotypic plasticity leads to tumor heterogeneity with implications for therapy. *PLoS Comput Biol* 2024; **20:** e1012003.

26 Hoenisch Gravel N, Nelde A, Bauer J, et al. TOF_{IMS} mass spectrometry-based immunopeptidomics refines tumor antigen identification. *Nat Commun* 2023; **14:** 7472.

27 Stein A, Simnica D, Schultheiß C, et al. PD-L1 targeting and subclonal immune escape mediated by PD-L1 mutations in metastatic colorectal cancer. *J Immunother Cancer* 2021; **9:** e002844.

28 Sahin U, Derhovanessian E, Miller M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* 2017; **547:** 222–26.

29 Perez-Riverol Y, Csordas A, Bai J, et al. The PRIDE database and related tools and resources in 2019: improving support for quantification data. *Nucleic Acids Res* 2019; **47:** D442–50.