A 3-week pause versus continued Bruton tyrosine kinase inhibitor use during COVID-19 vaccination in individuals with chronic lymphocytic leukaemia (IMPROVE trial): a randomised, open-label, superiority trial

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Summary

Background Chronic lymphocytic leukaemia is the commonest leukaemia and is associated with profound immunosuppression. Bruton tyrosine kinase inhibitors (BTKi) have revolutionised chronic lymphocytic leukaemia management; however, therapy impairs vaccine-induced immunity. We evaluated whether a 3-week pause of BTKi treatment improved spike protein receptor binding domain (RBD) immunity to SARS-CoV-2 booster vaccination while maintaining disease control.

Methods We performed an open-label, two-arm, parallel-group, randomised trial in secondary-care haematology clinics in 11 UK hospitals. Participants aged 18 years or older, diagnosed with chronic lymphocytic leukaemia, and currently taking BTKi therapy (frontline or relapsed setting) for at least 12 months were eligible. Participants were randomly allocated (1:1, by a centralised computer randomisation program, stratified by BTKi therapy line) to pause BTKi for 3 weeks, starting 6 days before their SARS-CoV-2 vaccination booster date, or to continue therapy as usual. Neither participants nor clinical staff were blinded but laboratory staff were. Intramuscular injection of either original BA.1 or original BA.4/5 bivalent mRNA vaccine (50 µg mRNA-1273 or 30 µg BNT162b2), or 5 µg protein-based Vidprevtyn Beta (Sanofi Pasteur, Lyon, France) were received according to the national vaccination programme schedule. The primary outcome measure was anti-spike-RBD-specific antibody titre 3 weeks after vaccination and analysis performed by intention to treat (as randomly allocated, irrespective of compliance) following trial completion. This trial is registered with ISRCTN, 14197181, and has been completed.

Findings Between Oct 10, 2022, and June 8, 2023, 99 individuals (71 [72%] male and 28 [28%] female, with 89 [90%] of White ethnicity) were randomly allocated to groups pausing (n=50 [51%]) or continuing (n=49 [49%]) their BTKi therapy, and followed up for 12 weeks. At 3 weeks after vaccination, the geometric mean anti-spike-RBD-specific antibody titre was 218·8 U/mL (SD 122·9) in the continue group and 153·4 U/mL (103·2) in the pause group, with geometric mean ratio 1·104 (95% CI 0·565–2·158, p=0·77) using a mixed-effects model. The only serious adverse event during the 12-week follow-up was the death of one participant in the pause group due to COVID-19 infection 2 months after randomisation.

Interpretation Although the study was slightly underpowered, the results suggest that pausing BTKi around the time of vaccination is not beneficial for immunity and should not be recommended in clinical practice.

Funding National Institute for Health and Care Research.

Introduction

Individuals with chronic lymphocytic leukaemia are among the most immunosuppressed in society, with a complex immune dysregulation.[1] Hence, these individuals had considerable morbidity and mortality (reported between 13% and 33%) at the start of the COVID-19 pandemic.[2,3] Booster vaccination has been used to overcome impaired immunity among immunocompromised individuals, with notable improvement, including in those with chronic lymphocytic leukaemia who do not require therapy.[4,5] In contrast, individuals taking covalent Bruton tyrosine kinase inhibitors (BTKi) continue to show the poorest responses despite having more than four vaccine doses.[6–8]

Bruton tyrosine kinase (BTK) plays an important role in the B-cell receptor signalling pathway in response to antigen recognition, resulting in B-cell activation and subsequently antibody affinity maturation.[9] Several

Research in context

Evidence before this study

Bruton tyrosine kinase inhibitor (BTKi) therapy impairs humoral immunity and is associated with infection-related morbidity and poor response to vaccination. We searched PubMed for studies published between Dec 10, 2021, and Oct 15, 2024, using the terms ([BTKi] AND [Vaccine] AND [COVID-19 or SARS-CoV-2]). We identified no randomised trials but found two observational studies suggesting a potential value to pausing BTKi therapy around the time of vaccination. These two studies found that a 3-week interruption in BTKi improved the SARS-CoV-2 antibody response to vaccination. The small number of participants and non-randomised data available from these studies make the results at high risk of bias, and thus the results cannot be extrapolated to inform health policy.

Added value of this study

To our knowledge, this study represents the first randomised trial of pausing BTKi monotherapy around the time of SARS-CoV-2 vaccination. The study found that pausing therapy for 3 weeks did not improve the anti-spike-RBD-specific antibody response at 3 weeks or 12 weeks after vaccination. Neither did pausing therapy improve the functionality of the antibody responses (measured by neutralisation) or the cellular response in participants taking BTKi. Additionally, there was a temporary increase in self-reported lymphadenopathy in those randomly allocated to pausing therapy.

Implications of all the available evidence

This study, although slightly underpowered, provides no evidence that pausing BTKi around the time of SARS-CoV-2 booster vaccination is beneficial for immunity, and it should not be recommended in clinical practice.

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covalent, irreversible BTKi drugs are licensed that block this pathway, preventing chronic lymphocytic leukaemia cell proliferation but at the expense of inhibiting normal B-cell responses.[10] T-cell immunity has been reported by some to improve in individuals treated with BTKi. Mechanisms proposed include possible immune reconstitution with disease control, improved synapse formation with antigen-presenting cells, and, for individuals taking BTKi ibrutinib, a possible off-target inhibition of IL-2 inducible tyrosine kinase (ITK) in $CD4^+$ Th2 cells.[11,12]

The available covalent BTKi molecules have a short half-life of the order of 4–6 h and are routinely paused around the time of surgery to reduce bleeding risk. Several observational studies have suggested that short interruptions in BTKi can improve antibody responses to vaccination. Existing estimates of BTK resynthesis vary, with rates of 14·5% and up to 31·4% per day reported in people with chronic lymphocytic leukaemia.[13,14] Full BTK restoration takes approximately 7–16 days after BTKi discontinuation; however, B-cell receptor signalling is correlated with the number of BTK sites occupied, and a return of function would precede full BTK restoration and be reported as early as 36 h following cessation of therapy.[14] Early observational data suggest that a short pause in BTKi therapy around the time of vaccination can improve immunity.[4,15,16] 3 weeks off therapy (2 weeks following vaccination) would also provide time for ongoing antigen presentation and germinal centre reactions to take place.[17] However, this practice has not been prospectively tested within a randomised trial, which has led to conflicting advice on whether pausing or continuing BTKi therapy around the time of vaccination is an effective intervention, and the safety of such an approach has not been evaluated. This relatively simple intervention could provide highly susceptible individuals with the opportunity of not only enhanced immunity but psychosocial benefit in navigating the era following the COVID-19 pandemic.

We hypothesised that a 3-week pause in covalent BTKi monotherapy starting 1 week before SARS-CoV-2 booster vaccination would enhance immunity following vaccination, without deterioration in disease control. We proposed that by pausing BTKi, both the strength and functional quality of the antibody response would improve as BTK signalling was restored. We addressed this hypothesis through antibody titre quantification against the receptor binding domain (RBD) of the SARS-COV-2 spike protein, and virus neutralisation against both the ancestral virus (B.1) and the variants of concern (VOCs) included in the administered vaccines (BA.1, B.1.351, and BA.5) at 3 weeks and 12 weeks following SARS-CoV-2 vaccination. In addition, cellular responses were examined through interferon gamma producing T-cell responses directed against ancestral B.1 and the XBB variant at 3 weeks, along with self-reported disease activity and a quality-of-life questionnaire.

Methods

Study design and participants

In this two-arm, parallel-group, multicentre, superiority, randomised controlled trial, 99 participants were recruited from haematology clinics in 11 National Health Service hospitals in England and Wales (appendix p 1). The study was approved by the Leeds East Research Ethics Committee and Health Research Authority. The full study protocol has previously been published,[18] with no substantial changes having been

See Online for appendix

made since. No deviations occurred that were deemed to have a meaningful effect on the results of the study.

Eligible participants were aged 18 years or older, had a diagnosis of chronic lymphocytic leukaemia, and had been prescribed a BTKi (ibrutinib or acalabrutinib) for at least 12 months and anticipated to take it during the next 4 months. Any performance status and comorbidity was permitted. Participants had to be in complete remission (including complete remission with incomplete marrow recovery) or partial remission (including nodular partial remission or partial remission with lymphocytosis), or have stable disease by International Workshop on Chronic Lymphocytic Leukaemia response criteria, and had to be willing to accept either trial group allocation, ie, to pause or continue their existing BTKi therapy for 3 weeks. Eligible participants were considered able to pause BTKi therapy for 3 weeks without the risk of substantial increase in disease activity and due to receive a SARS-CoV-2 booster vaccination as part of the autumn, 2022, or spring, 2023, UK COVID-19 vaccination programme.

Participants were excluded if they had been diagnosed with alternative conditions requiring treatment with BTKi, active solid organ cancer, or Richter's transformation; or had received alternative immune suppression in the last 3 months (azathioprine, prednisolone, ciclosporin, mycophenolate, BCL-2 inhibitors, methotrexate, and biological agents), radiotherapy or cancer chemotherapy in the last 6 months, anti-CD20 monoclonal in the last 18 months, or COVID-19 spike monoclonal antibody therapy or immunoglobulin replacement therapy in the last 6 months. Informed, written consent was obtained before randomisation.

Randomisation and masking

Participants were randomly allocated (1:1) either to continue taking BTKi as usual or to have a 3-week pause (1 week before and 2 weeks after their SARS-CoV-2 booster vaccination). Randomisation was done using a centralised, validated computer randomisation program through a secure (encrypted) web-based service, provided by the Oxford Clinical Trials Research Unit. Eligible participants were randomly allocated once a date for their SARS-CoV-2 booster vaccination was known, and were notified of their allocated group immediately either in person or by telephone. The randomisation used permuted blocks of varying size prepared by a study statistician, stratified on first line or subsequent line of therapy for chronic lymphocytic leukaemia to ensure balance across the two groups. Due to the nature of the intervention, the participants and the clinical team were not masked to participants' allocation. Trial statisticians were also not masked, whereas those analysing the study samples were masked. Previous SARS-CoV-2 infection status was established by measuring antibody response to SARS-CoV-2 nucleoprotein at baseline and included in the statistical analysis.

Procedures

Participants' baseline blood samples and questionnaires with global disease assessment were obtained at least 1 week before vaccination (clinic). Participants received either 50 µg mRNA-1273 bivalent BA.1 or BA.4/5 mRNA (Spikevax; Moderna, Cambridge, MA, USA), or 30 µg bivalent BNT162b2 BA.1 or BA.4/5 mRNA (Comirnaty [Pfizer–BioNTech]; Pfizer, New York, NY, USA, and BioNTech, Mainz, Germany) or 5 µg SARS-CoV-2 beta protein vaccine (VidPrevtyn Beta; Sanofi, Paris, France) intramuscularly. Those randomly allocated to pause BTKi stopped 1 week before vaccination (delivered on day 7) and remained off BTKi for a further 14 days before restarting. Those continuing BTKi took their usual daily dose throughout the study, including the day of vaccination. Concomitant medication including antiviral therapies were continued throughout. Participants could resume BTKi against trial allocation if clinically indicated, including for disease flare. Outcomes were assessed at 3 weeks and 12 weeks after the booster vaccination date (clinic). Antibody measurements were undertaken at the University of Birmingham Clinical Immunology Service using the quantitative Elecsys immunoassay (Roche Diagnostics, Basel, Switzerland). Neutralising activity was measured using a pseudotyped virus neutralisation assay in which the SARS-CoV-2 spike proteins (B.1, B.1.351, BA.1, and BA.5) are expressed on the surface of HIV particles bearing a luciferase marker gene. T-cell responses (cellular immunity) were assessed using the Human IFN-γ and IL-2 FluoroSpot Plus Kit (Mabtech, Nacka Strand, Sweden) with different peptide pools (Wuhan PM-WCPV-S-2 and XBB.1.5 PM-SARS2-SMUT15-1; JPT Peptide Technologies, Berlin, Germany). Self-reported quality of life was assessed using the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire for Chronic Lymphocytic Leukaemia 17 (EORTC-QLQ-CLL17).[19] Global assessment of disease activity was assessed at 3 weeks and 12 weeks using a 0–10 numerical rating scale for the past week and, additionally, whether individuals had a fever of 37·5°C or higher or noticed that new or enlarged lymph nodes had occurred; clinical blood measures (haemoglobin, platelet levels, lymphocyte and lactate dehydrogenase levels, presence of new prolymphocytes, smear cells, or lymphocytosis); and self-report adherence. Participants were not excluded if they tested positive for SARS-CoV-2. Voluntary additional blood samples were also taken on the day of vaccination and 1 week after vaccination for future research studies.

Outcomes

The primary outcome measure was RBD antibody titres 3 weeks after SARS-CoV-2 booster vaccination. Secondary

outcomes were: RBD titres at 12 weeks after the vaccination, neutralisation and T-cell response against the ancestral virus and VOCs at 3 weeks after vaccination; participant assessment of disease activity and change since baseline, 3 weeks and 12 weeks after vaccination; disease flare-up, actions taken to deal with this, and effects on quality of life at 3 weeks and 12 weeks after vaccination; and self-reported adherence to trial allocation at 3 weeks after vaccination. Serious adverse events were also recorded from the booster vaccination to 12 weeks after vaccination.

Statistical analysis

The sample size calculation was based upon detecting a difference in (standardised) effect size of 0·65 SD, with 90% statistical power at two-sided 5% significance level. This difference in effect size is equivalent to detecting a mean difference of 0·97 units on the $\log_{10}$ scale, ie, a mean higher antibody response in the BTKi suspension group. This requirement necessitates data from 102 participants; we set the study (target) sample size at 120 participants to allow for 15% missing data.

The principal analysis was performed once the last participant completed follow-up, on the as-randomised (intention-to-treat) population with available outcome data in their randomised groups, regardless of adherence. No interim analyses were planned or conducted.

Antibody data were $\log_{10}$ transformed to normalise the distribution before analysis. The anti-RBD antibody levels were summarised descriptively at baseline and at 3-week and 12-week follow-up after the SARS-CoV-2 booster vaccination. The differences between the study groups were estimated using a mixed-effects regression model, allowing for repeated measures clustered within participants. The model was adjusted for the randomisation factor (first line or subsequent line of therapy) and previous infection status obtained from pre-vaccination anti-nucleocapsid antibodies and type of SARS-CoV-2 booster vaccine received as fixed effects. A treatment by time (as a categorical variable) interaction was included, and the model used an unstructured covariance matrix and maximum likelihood estimation. The adjusted mean differences between the groups are presented, together with 95% CIs and p values. Supplemental analyses explore the time effect between the last COVID-19 vaccination dose and the dose given in the IMPROVE trial. The effect of non-compliance to the randomised intervention was explored using per-protocol analyses.

The same model as for the primary outcome was used to analyse the 12-week anti-RBD antibody outcome. Similar supportive analyses were performed for 12-week neutralisation data. Overall, 50% and 90% neutralisation by the ancestral virus and VOCs, along with T-cell spike response by subtype (ancestral virus and VOCs), were analysed in a similar manner to anti-RBD antibody outcome (model adjustments were simplified given sample subsets were used). Other secondary outcomes were analysed using linear regression models for participant-reported outcomes (EORTC-QLQ-CLL17) with model adjustment for baseline values.

Exploratory primary outcome subgroup analysis was undertaken comparing the consistency of the treatment effect between those on their first line of BTKi therapy and those on subsequent lines (two to four lines) using treatment-by-subgroup interactions. A post-hoc primary outcome analysis was completed looking just at the subset of participants who seroconverted (RBD antibody titres >0·8 U/mL at baseline according to manufacturer protocol) when entering the study. A planned complier average causal effect analysis along with exploration of the effect of missing data on the primary outcome was not carried out given the very high level of compliance and data available for the primary outcome.

Serious adverse events were summarised by treatment group; the proportion of participants with at least one serious adverse event was assessed descriptively. Missing data were described with reasons given where available; the number and percentage of individuals in the missing category were presented by treatment

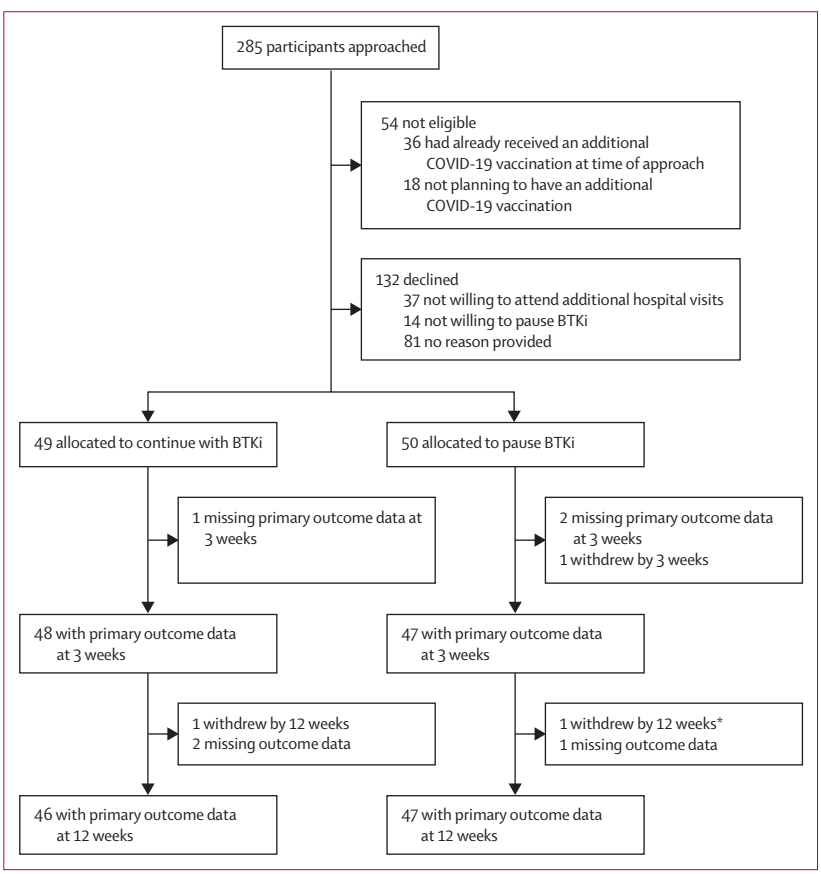

Figure: **Trial profile**
BTKi=Bruton tyrosine kinase inhibitor. *One participant withdrawn before the week 12 timepoint died due to complications from COVID-19 infection within 12 weeks of randomisation.

	Continue BTKi group(n=49)	Pause BTKi group(n=50)
Age, years	71·1 (7·8)	69·8 (9·7)
Biological sex		
Male	35 (71%)	36 (72%)
Female	14 (29%)	14 (28%)
BMI	27·8 (3·7)	28·4 (5·4)
Time on BTKi, days	920 (665–1529)	942 (620–1836)
Ethnicity		
White—English, Welsh, Scottish, Northern Irish, or British	45 (92%)	44 (88%)
White—any other White background	1 (2%)	1 (2%)
Asian or Asian British—Indian	2 (4%)	1 (2%)
Asian or Asian British—Pakistani	0	1 (2%)
Black, African, Caribbean, or Black British—African	0	1 (2%)
Black, African, Caribbean, or Black British—any other Black, African, or Caribbean background	1 (2%)	2 (4%)
Smoking status		
Never smoked	29 (59%)	23 (46%)
Ex-smoker	19 (39%)	21 (42%)
Ex-smoker and current vaper	0	2 (4%)
Current smoker	1 (2%)	4 (8%)
Comorbidities		
Diabetes	4 (8%)	6 (12%)
Hypertension	17 (35%)	14 (28%)
Ischaemic heart disease	3 (6%)	5 (10%)
Congestive cardiac failure	2 (4%)	2 (4%)
Asthma	0	3 (6%)
Chronic obstructive pulmonary disease	2 (4%)	4 (8%)
Stroke	2 (4%)	1 (2%)
High cholesterol	8 (16%)	9 (18%)
None of the above	23 (47%)	25 (50%)
BTKi first-line or subsequent therapy		
First-line	31 (63%)	32 (64%)
Subsequent	18 (37%)	18 (36%)
Total lines of BTKi		
1	31 (63%)	32 (64%)
2	13 (27%)	15 (30%)
3	3 (6%)	2 (4%)
4	2 (4%)	1 (2%)
Chronic lymphocytic leukaemia medications in past 24 months, but stopped >12 months before study		
Rituximab	1 (2%)	0
Fludarabine	1 (2%)	0
Cyclophosphamide	1 (2%)	0

(Table 1 continues in next column)

group. Clinical blood measures and adherence were summarised.

The study is registered with ISRCTN (ISRCTN14197181) and has been completed.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit for publication.

Results

Between Oct 10, 2022, and June 8, 2023, 285 patients were approached and 99 were recruited by the end of the funded recruitment period, reflecting the UK Department of Health vaccination campaign window (figure). Participants were randomly assigned to pause (n=50) or continue (n=49) BTKi therapy. One participant was withdrawn in the pause group by site before the primary endpoint at 3 weeks after randomisation because they decided not to proceed with vaccination. Additionally, two other participants in the pause group and one participant in the continue group were missing 3-week primary outcome data. Two participants (one each in the continue and pause group) were withdrawn or died before providing 12-week blood samples (one due to undiagnosed metastatic cancer at enrolment, and one due to infection).

The baseline characteristics were similar in both groups of the study (table 1). The cohort mean age was 70·5 years (SD 8·8), 71 (72%) of the 99 participants were male and 28 (28%) female, and 51 (52%) participants were taking ibrutinib therapy whereas 48 (48%) were taking acalabrutinib at 100 mg twice daily. Ibrutinib was prescribed at the recommended 420 mg daily dosage in 43 (84%) participants, with reduced dosages of 280 mg daily in five (10%) and 140 mg daily in three (6%) of the 51 participants taking ibrutinib. 63 (64%) participants were receiving BTKi as first-line therapy. Of the 36 (36%) participants receiving BTKi as a subsequent line of therapy, 28 (78%) were taking it as second line. The median time on drug at the point of study enrolment was 933 days (n=99). Serum IgG, IgA, and IgM concentrations were similar between the two study groups.

The median number of COVID-19 vaccine doses received before enrolment was five (IQR 5–6), with 92 (94%) participants having received at least four doses (table 1). 91 (92%) participants received an mRNA booster vaccine, whereas seven (7%) received a protein-based booster vaccine, and for one participant the booster vaccine type was unknown (table 2). Adherence to group allocation was high, with 46 (92%) in the pause group and 49 (100%) in the continue group reporting compliance. Participants were not excluded from the analysis for non-compliance (except the primary outcome per-protocol analysis). Protocol deviations were categorised and balanced across treatment allocation (appendix p 3).

The geometric mean RBD antibody titres at 3 weeks after the booster vaccine dose were 218·8 U/mL (SD 122·9) in the continue group and 153·4 U/mL (103·2) in the pause group, with a geometric mean ratio (GMR) of 1·104 (95% CI 0·565–2·158, p=0·77; mixed-effects model adjusted for baseline SARS-CoV-2 values in the two groups, nucleocapsid status at baseline, booster vaccine type, and first or subsequent BTKi treatment line). At 12 weeks, again no difference was observed in RBD antibody titres between the two study groups (177·6 U/mL [95·3] in the continue group *vs* 122·4 U/mL [92·5] in the pause group), with a GMR of 1·037 (0·529–2·035, p=0·91; table 3).

A detectable antibody response (defined by RBD antibody titre ≥0·8 U/L) to SARS-CoV-2 RBD was present in 67 (68%) participants at baseline. At 3 weeks, this proportion was similar to at baseline (appendix p 3). The response rate was 33 (69%) of 48 participants who continued BTKi and 32 (68%) of 47 who paused BTKi therapy at 3 weeks after the vaccine dose (appendix p 3). Evidence of natural infection, measured by a detectable nucleocapsid antibody response, was also similar in both study groups: nine (18%) participants in the continue group versus ten (20%) in the pause group at baseline, with an additional two participants positive in the pause group at the 12-week timepoint (appendix p 3). The 3-week antibody titres were lower overall in those who were receiving BTKi as first-line therapy (n=62; geometric mean 136·9 U/mL [SD 99·7]) compared with those receiving it as a subsequent therapy line (n=33; 318·6 U/mL [136·0]; appendix pp 3–4).

Given that one-third of participants continued to have no detectable antibody responses, RBD antibody titre was reassessed in post-hoc analyses and compared among those participants with a detectable response at baseline; again, no difference was observed between the two study groups at 3 weeks after the vaccine dose (geometric mean antibody titre 2708·6 IU/mL [SD 25·2] in the continue group and 2310·5 [21·1] IU/mL in the pause group; GMR 0·746 [95% CI 0·173–3·218], p=0·694; appendix pp 3–4). Other analyses of the primary outcome had similar findings (appendix pp 3–4).

To assess whether pausing BTKi improved the quality of antibody response, neutralisation assays were performed for ancestral, B1.351, and omicron VOCs, to reflect the immunogenic components among the vaccines received. Although neutralisation titres were consistently higher among antibody responders 3 weeks after vaccination than at baseline, interruption of treatment did not improve neutralisation of the ancestral B.1 virus at 3 or 12 weeks (GMR 1·073 [95% CI 0·626–1·837] and 0·647 [0·378–1·109]) compared with those who continued on BTKi. Neutralisation to VOCs was also diminished in comparison with B.1 and again showed no difference in either study group (table 4).

Finally, an assessment of cellular immunity showed no difference in interferon gamma response to B.1 peptide stimulation between the two study groups: 454 spot-forming cell units (SFCU) per 10^6 cells (SD 6) at 3 weeks in the continue group compared with 433 SFCU per 10^6 cells (4) in the pause group (GMR 1·366 [95% CI 0·898–1·988], p=0·15). A reduced but similar response was also observed in both groups for the XBB variant: 322 SFCU per 10^6 cells (5) at 3 weeks in the continue group compared with 231 SFCU per 10^6 cells (4) in the pause group (GMR 1·327 [0·851–2·070], p=0·21; appendix p 5).

The presence of prolymphocytes on blood film, the total lymphocyte count, and lactate dehydrogenase values

	Continue BTKi group(n=49)	Pause BTKi group(n=50)
(Continued from previous column)		
Current disease stage by International Workshop on Chronic Lymphocytic Leukemia criteria (Binet system)		
A	38 (78%)	43 (86%)
B	5 (10%)	4 (8%)
C	6 (12%)	3 (6%)
BTKi taken		
Ibrutinib	22 (45%)	29 (58%)
Acalabrutinib	27 (55%)	21 (42%)
Usual ibrutinib dose		
140 mg	1/22 (5%)	2/29 (7%)
280 mg	4/22 (18%)	1/29 (3%)
420 mg	17/22 (77%)	26/29 (90%)
Usual acalabrutinib dose		
100 mg twice daily	27/27 (100%)	21/21 (100%)
Number of previous COVID-19 vaccinations*		
Median	5 (4–6)	5 (5–6)
Mean and range	5·0 (1·1), 1–7	5·1 (0·9), 3–6
≥4 vaccinations	46 (94%)	46 (94%)
Immunoglobulin levels		
IgG (≥6·0 g/L)	25 (51%)	27 (54%)
IgA (≥0·8 g/L)	25 (51%)	27 (54%)
IgM (≥0·5 g/L)	11 (22%)	15 (30%)

Data are mean (SD), median (IQR), n (%), n/N (%), or mean (SD), range. BTKi=Bruton tyrosine kinase inhibitor. *One participant did not provide information on number of previous vaccinations; all other baseline data were complete.

Table 1: **Participant characteristics at study entry**

	Continue with BTKi group (n=49)	Pause BTKi group (n=50)
Bivalent Pfizer vaccine	42 (86%)	43 (86%)
Bivalent Moderna vaccine	2 (4%)	4 (8%)
Sanofi vaccine	5 (10%)	2 (4%)
Unknown or did not receive booster vaccine*	0	1 (2%)

Data are n (%). BTKi=Bruton tyrosine kinase inhibitor. *One participant in the group continuing with BTKi missed week 3 and did not provide information at this timepoint.

Table 2: **SARS-CoV-2 booster vaccines received**

	Continue with BTKi group (n=49)		Pause BTKi group (n=50)		Geometric mean ratio, mixed-effects model*	p value, mixed-effects model
	n	Geometric mean, U/mL	n	Geometric mean, U/mL		
Baseline	49	133·0 (82·2)	50	76·8 (97·1)	..	..
3 weeks	48	218·8 (122·9)	47	153·4 (103·2)	1·104 (0·565–2·158)	0·77
12 weeks	46	177·6 (95·3)	47	122·4 (92·5)	1·037 (0·529–2·035)	0·91

Data are n, mean (SD), ratio (95% CI), or p. BTKi=Bruton tyrosine kinase inhibitor. *Presenting a model adjusted for baseline values to account for the difference between groups at baseline, BTKi treatment line (first or subsequent), COVID-19 infection status at baseline, and booster type as fixed effects, and with a treatment by time interaction.

Table 3: **Primary outcome at baseline, 3 weeks, and 12 weeks**

	Continue with BTKi group (n=49)		Pause BTKi grpup (n=50)		Geometric mean ratio, mixed-effects model*	p value, mixed-effects model
	n	Geometric mean, U/mL	n	Geometric mean, U/mL		
B.1						
Baseline	47	231 (7·3)	50	200 (6·3)	..	..
3 weeks	46	377 (8·3)	47	359 (8·4)	1·073 (0·626–1·837)	0·80
12 weeks	46	346 (8·1)	47	228 (8·0)	0·647 (0·378–1·109)	0·11
B.1.351						
Baseline	47	175 (6·1)	50	154 (5·2)	..	..
3 weeks	46	319 (8·0)	47	293 (7·6)	0·986 (0·581, 1·671)	0·96
12 weeks	46	185 (5·5)	47	140 (4·9)	0·717 (0·423, 1·216)	0·22
BA.1						
Baseline	47	90 (4·0)	50	131 (5·4)	..	..
3 weeks	46	169 (6·1)	47	194 (7·3)	0·832 (0·473, 1·463)	0·52
12 weeks	46	129 (4·7)	47	130 (6·0)	0·661 (0·376, 1·163)	0·15
BA.5						
Baseline	47	106 (4·1)	50	125 (4·5)	..	..
3 weeks	46	171 (6·2)	47	159 (5·3)	0·802 (0·510, 1·259)	0·34
12 weeks	46	124 (4·6)	47	117 (4·5)	0·700 (0·445, 1·101)	0·12

Data are n, mean (SD), ratio (95% CI), or p. BTKi=Bruton tyrosine kinase inhibitor. *Model adjusted for baseline values to account for the difference between groups at baseline, BTKi treatment line (first or subsequent), COVID-19 infection status at baseline, and booster type as fixed effects, with a treatment by time interaction.

Table 4: **Analysis of the neutralisation outcome data**

were similar between the two groups at 3 weeks or 12 weeks from baseline (appendix pp 5–6). The self-reported quality of life questionnaire (EORTC-QLQ-CLL17) and the global disease activity rating were similar between both groups of the study at all timepoints (appendix pp 6–7). At week 3, more participants in the pause group than the continue group reported fatigue (mean difference mixed model 0·170 [95% CI –0·005 to 0·346], p=0·058), but by 12 weeks this outcome was similar again in both groups. Self-reported lymphadenopathy was reported in four (9%) of 47 participants in the pause group at 3 weeks after vaccination, which reduced to one (2%) participant by 12 weeks (appendix pp 4–5). In comparison, no participants in the continue group reported any lymphadenopathy. Two participants sought National Health Service care before the 3-week follow-up and both were in the pause group. No participants required hospital admission. Between 3 and 12 weeks, one participant was admitted and treated for infection. Two participants in the pause group resumed therapy earlier than the 3-week period. One participant in the pause group died on study due to COVID-19 infection 2 months after randomisation. There were no other serious adverse events during the 12-week follow-up (appendix pp 7–8).

Discussion

A 3-week pause in BTKi therapy did not improve the antibody titre elicited by SARS-CoV-2 booster vaccination at 3 weeks or 12 weeks after vaccination. Instead, a highly heterogeneous response was observed with similar proportions of non-responders in both groups. The majority of participants had already received at least four previous COVID-19 vaccination doses, which indicates that a plateau had been reached where non-responders were unlikely to seroconvert. The heterogeneous ability of participants taking BTKi to respond to vaccination is also likely to account for the higher baseline titre observed in the continue group compared with those who paused. Somewhat surprisingly, antibody titres were also lower in those receiving first-line BTKi therapy. This is in contrast to data showing infection risk to be higher in those receiving BTKi in a relapsed refractory setting,[20] but, together with the heterogeneous responses observed in the cohort, highlights the need for greater understanding as to the determinants of an immune response in this cohort and the mechanisms by which some participants on BTKi were able to mount an antibody response whereas others were not.

Implementing a temporary cessation of immunosuppressive medication has previously been reported to improve immunity. In chronic lymphocytic leukaemia, Tomasulo and colleagues recently reported a 3·45-fold higher median antibody titre in a non-randomised study of 12 participants who paused BTKi (median 5·5 days before 10·5 days after vaccination) compared with 33 participants who continued.[15] The VROOM study for participants taking methotrexate for rheumatoid arthritis and other conditions also found a benefit when therapy was paused for only 2 weeks in a randomised setting, giving further rationale for this relatively simple intervention to be explored.[21] BTKi is also commonly paused for individuals admitted to hospital with infection with the expectation that this could aid recovery.[22] Given the extent of immune suppression and infection morbidity associated with taking BTKi,[23] the relative benefit to adopting this pause strategy at the time of vaccination has become pertinent. This study, however, has found no evidence of such benefit and is the first to

test this hypothesis in a randomised trial setting for individuals with chronic lymphocytic leukaemia.

The variability in antibody responses and absence of benefit to pausing therapy is intriguing. The short half-lives of the two BTKi drugs investigated here (ibrutinib and acalabrutinib) and existing receptor occupancy data would support drug reversibility and return of function to the B-cell receptor signalling pathway after 1 week off drug.[13,14] It is therefore somewhat surprising that no difference was observed between groups, but this suggests that BTK inhibition is not the only limiting factor to antibody generation among this group; there are several potential reasons why this could be. First, chronic lymphocytic leukaemia immune suppression is complex and multifactorial, with other components of the immune response, aside from the BCR signalling contributing to vaccine responsiveness.[1,24] Second, it is not clear in participants taking BTKi therapy whether B-cell immune reconstitution arises as disease is controlled and whether the depth of response to BTKi therapy could be influencing antibody titre. Third, the number of vaccine doses might be influential; virtually all participants had completed their three-dose primary vaccine course. Indeed, in many immunocompromised participants, a third primary dose became standardised as improvement was seen with a subsequent dose.[4–6,25] Our data suggest that a plateau is then reached whereby there is no further seroconversion with additional doses, even in the absence of BTKi. In contrast, many participants had detectable antibody responses despite taking continuous therapy. For those who were able to generate an antibody response, an increase in titre was observed at 3 weeks and 12 weeks after vaccination regardless of whether they paused or continued the drug, and the quality of the antibody response, measured through viral neutralisation assays, also improved. This finding suggests that in a proportion of participants the functionality of the antibodies produced was optimal, despite the participants being on continuous therapy. It remains to be seen whether this finding reflects a high affinity response that has undergone affinity maturation and class switching and, if so, how this is feasible while taking a covalent BTKi drug.[17]

A limitation of this study is the variety of vaccines participants received. The provision of vaccines was based on the UK Department of Health recommendation and stock availability at vaccination hubs. Although this variety could have potentially introduced different immunogenicity, nearly all participants received an mRNA vaccine that contained the antigen sequence homology to the ancestral B.1 virus, for which the primary outcome, RBD antibody testing, was specific. Seven participants received the protein-based vaccine from the later B.1.351 variant, slightly more in the continue than pause group. Recruitment was lower than targeted and the trial was slightly underpowered, so the results reflect increased uncertainty and therefore a difference in antibody response cannot be completely ruled out, particularly given the heterogeneity of participant responses and vaccinations received.

Interrupting therapy did not affect quality of life and concordance with advice to pause therapy was excellent, with only two participants restarting therapy during the initial 3-week period. However, consistent with recognised withdrawal symptoms,[26] more participants in the pause group self-reported lymphadenopathy, although other features of disease flare, including lactate dehydrogenase levels, remained similar in both groups. Furthermore, no participants required medical intervention for lymphadenopathy and they self-managed their symptoms, with normalisation reported by 12 weeks.

Vaccination strategies for the immunocompromised remains a challenge and this is particularly true where therapy is required in a continuous manner. COVID-19 represented a neo-antigen exposure in 2019, when many participants were already taking BTKi. The impact of BTKi on vaccine responses that rely on recall from long-lived memory B and plasma cells could be less pronounced and emphasis should still be placed on optimising vaccination before commencing therapy.[27] It is also unclear how translatable these findings are to other types of vaccine platforms, particularly where previous memory B-cell responses might already be present, such as with varicella zoster vaccination.[28] An exploratory outcome had been planned to assess the effect on co-administration of influenza vaccination, but this was not possible due to a low number of participants who received their COVID-19 and influenza vaccination at the same time during the study.

The data also support robust cellular immune responses in both groups of the study, with incremental improvement following vaccination. This improvement included responses to the XBB variant, the sequence for which was not a component of the vaccines received by participants in this study. In support of this finding, several studies have shown global improvement to cellular immunity on BTKi.[11,29] In the context of COVID-19 vaccination, quantification of antigen-specific T-cell responses by flow cytometry have also been shown in participants taking BTKi, and cellular responses appear to be similar to those of healthy controls.[30] This finding augurs well for cross-protection against VOCs, provides additional protection for those without humoral responses, and supports the use of booster vaccinations for individuals on BTKi to reduce the hospitalisations and mortality rates observed with earlier variants.[22]

In conclusion, this randomised trial found no benefit in pausing BTKi therapy around the time of vaccination with a SARS-CoV-2 booster. As such, in clinical practice, individuals should not be recommended to pause their BTKi therapy to enhance immunity to vaccination. The results highlight the importance of testing treatment interventions in a powered, randomised setting but also

the need for more fundamental science to consider the drivers of the heterogeneous responses observed. This vulnerable group continues to be at risk from infections and necessary precautions should be adopted where available, such as monoclonal antibody prophylaxis.

Contributors

HMP, PEMP, JC, and AA designed the study. HMP, PEMP, JAC, AA, PM, LD, and VB obtained the funding. HMP, JAC, VB, and NPe wrote the manuscript. HMP, NPe, PEMP, GT, NP-J, TAE, FAW, AMS, CH, SP, DJM, NM-C, SJ, EH, and SS recruited participants and investigated. TR, MH, RH, and BJW performed the experiments. VB, AF, GP, and GV curated the data and project administration. JAC and NPe verified and analysed the data. LD represented the trial patient advisory group. All authors had full access to the data, reviewed the manuscript, and had final responsibility for the decision to submit for publication.

Declaration of interests

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Data sharing

The de-identified data used in this study can be shared after approval by the ethics authority and on request to the corresponding author.

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