Effect of rituximab on immune status in children with mature B-cell non-Hodgkin lymphoma: a prespecified secondary analysis of the Inter-B-NHL Ritux 2010 trial

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Summary

Background Survival of children and adolescents with high-risk, mature B-cell non-Hodgkin lymphoma is improved by the addition of rituximab to chemotherapy. The effect of rituximab on immune reconstitution after therapy has not been well described. Herein, we evaluate the immune effects of the addition of rituximab to intensive chemotherapy, a prespecified secondary aim of the Inter-B-NHL Ritux 2010 trial.

Methods The Inter-B-NHL Ritux 2010 trial was an international, open-label, randomised, phase 3 trial in children (age 6 months to 18 years) with high-risk, mature B-cell non-Hodgkin lymphoma, comparing chemotherapy alone or chemotherapy with rituximab. Measures of immune status were completed at baseline, 1 month from the end of treatment, and 1 year from the start of therapy, and yearly thereafter until normalised. For this secondary analysis, we report on the proportions of patients with low lymphocyte counts and immunoglobulin concentrations at these timepoints with total lymphocyte count, B-cell count, and IgG concentration as the main endpoints. Other endpoints of interest included exposure to immunoglobulin replacement therapy and vaccine serologies. The population assessed for immune endpoints was the eligible per-protocol population with at least one immune parameter at one timepoint. Comparisons of immune status were made between the randomised treatment groups. Safety in the post-therapy period was assessed in the population eligible for the immunity study who were followed up at least 3 months after the end of treatment and without cancer-related events. The Inter-B-NHL Ritux 2010 study was registered with ClinicalTrials.gov, NCT01516580; status completed, with analyses of secondary aims ongoing.

Findings From Dec 19, 2011, to June 13, 2017, 421 patients (344 [82%] boys and 77 [18%] girls; mean age was 8·8 years [SD 4·1]) were enrolled and had immune data at baseline during follow-up, or both. The study population included randomly assigned patients (n=289) and a non-randomised cohort enrolled after the planned interim analysis (n=132). At baseline, 99 (34%) of 290 patients with available data (excluding patients with bone marrow disease with peripheral blast cells) had lymphopenia, and 178 (48%) of 368 had hypogammaglobulinemia. 1 month from the end of therapy, patients who received chemotherapy with rituximab were more likely than those who received chemotherapy alone to have lymphopenia (86 [81%] of 106 *vs* 53 (60%) of 89, odds ratio [OR] 2·92 [95% CI 1·53–5·57], p=0·0011), B-cell lymphopenia (72 [96%] of 75 *vs* 36 [64%] of 56, OR 13·33 [3·71–47·84], p<0·0001), and hypogammaglobulinemia (67 [71%] of 95 *vs* 37 [47%] of 79, OR 2·72 [1·45–5·07], p=0·0017). Differences remained at 1 year for hypogammaglobulinemia only (52 [55%] of 94 *vs* 16 [25%] of 63, OR 3·64 [1·81–7·31], p=0·0003). Patients in the chemotherapy with rituximab group were more likely than those in the chemotherapy group to receive immunoglobulin replacement (26 [16%] 164 *vs* nine [7%] of 158, hazard ratio [HR] 2·63 [95% CI 1·23–5·62], p=0·010), mainly due to low immunoglobulin concentration. In the combined treatment groups, including non-randomly assigned patients, the proportion of patients who had loss of protective serologies to a vaccine preventable infection varied from four (9%) of 47 for polio to 21 (42%) of 50 for *Streptococcus pneumoniae* (pneumococcus). One patient (chemotherapy with rituximab group) had a life-threatening infectious event of polymicrobial bacterial sepsis reported 2 months after the final chemotherapy administration.

Interpretation Children with high-risk mature B-cell non-Hodgkin lymphoma receiving chemotherapy with rituximab were at risk of prolonged hypogammaglobulinemia, although severe infections were rare. Strategies for immunoglobulin replacement and revaccination are needed.

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Research in context

Evidence before this study

We considered the evidence from studies in patients with lymphoma and rituximab exposure and in paediatric patients treated with rituximab for other indications. In preparation of this Article, we searched PubMed on Jan 15, 2023, with no date or language restrictions, using the terms "rituximab", "hypogammaglobulinemia" and "lymphoma". Existing paediatric cancer data suggested that the addition of rituximab to chemotherapy did not substantially increase risks of infectious toxicity during the period of active therapy. Population-based data have shown that exposure to rituximab in the treatment of lymphoma is associated with increased risks of hypogammaglobulinemia and infections in adult patients lymphoma in long-term follow-up studies. Data are scarce on immune status and infection risk after therapy in paediatric patients treated with rituximab for lymphoma. The Inter-B-NHL Ritux 2010 study was an international, randomised, phase 3 trial in children and adolescents (aged 6 months to 18 years) with high-risk mature B-cell non-Hodgkin lymphoma, comparing event-free survival between those receiving intensive chemotherapy or intensive chemotherapy with rituximab. A prespecified, secondary aim of the study was an evaluation of the immune effects of rituximab therapy in paediatric patients after completion of intensive therapy.

Added value of this study

The Inter-B-NHL Ritux 2010 trial established the use of rituximab in addition to intensive chemotherapy as a standard treatment for children with high-risk mature B-cell non-Hodgkin lymphoma, and rituximab for this indication has been approved by both the US Food and Drug Administration (December, 2021) and the European Medicines Agency (March, 2020). The present secondary analysis adds value to the existing literature by indicating the risks associated with the use of rituximab in this context, specifically in terms of the risk of prolonged hypogammaglobulinemia. This study provides some reassurance that despite prolonged hypogammaglobulinemia, severe infections after completion of therapy are rare. This study also provides data on the clinical use of immunoglobulin replacement therapy in this setting and, in a subset of children, the rates of protective vaccine serologies at baseline and post-therapy. We provide the first large dataset on the parameters of immune function in this patient group. Furthermore, our data suggest that a small proportion of patients might have undiagnosed primary immunodeficiency, but that baseline immunological parameters alone cannot identify these patients.

Implications of all the available evidence

The present data inform on the need for awareness and monitoring of hypogammaglobulinemia after completion of therapy in paediatric patients with high-risk mature B-cell non-Hodgkin lymphoma. All the available evidence, including that provided in this study, suggest that patients with lymphoma treated with rituximab require immune follow-up care after therapy. The data from this study highlight existing research gaps, including the need for further data to inform the optimal use of immunoglobulin replacement therapy and vaccination strategies, and the need for systems to identify children with high-risk mature B-cell non-Hodgkin lymphoma who might have primary immunodeficiency.

Introduction

Rituximab is a chimeric monoclonal antibody directed at CD20 and is now a standard component of therapy in both adult and paediatric patients with diffuse large B-cell and Burkitt lymphoma.[1–5] In meta-analyses of studies in adult patients with lymphoma, the addition of rituximab to chemotherapy was not associated with an increase in severe infectious toxicity during therapy.[6,7] In children and adolescents (aged 6 months to 18 years) with high-risk mature B-cell non-Hodgkin lymphoma, the randomised Inter-B-NHL-Ritux 2010 trial showed that, during therapy, life-threatening febrile neutropenia (Common Terminology Criteria for Adverse Events [CTCAE] grade 4) occurred in 19 (12%) of 162 patients in the chemotherapy with rituximab group versus ten (7%) of 153 in the chemotherapy alone group (p=0·11). Grade 4 or 5 (life-threatening or fatal) infectious events occurred in 30 (19%) patients in the chemotherapy with rituximab group versus 17 (11%) in the chemotherapy group (p=0·070).[1]

The longer term effect of adding rituximab to chemotherapy for patients with mature B-cell lymphoma with regard to the risk of secondary immunodeficiency is less clear. A study of adults with five common cancers, who were in remission at 1 year from the time of diagnosis and followed up for 1–10 years, reported that patients with diffuse large B-cell lymphoma had higher risks of infection, autoimmune diseases, and immunodeficiencies than those treated for other cancers.[8] In patients with diffuse large B-cell lymphoma, the risk of humoral immunodeficiency was significantly increased in patients treated in the period when rituximab was a standard component of therapy, compared with those treated in the pre-rituximab period.[8]

In all age groups, rituximab causes a rapid depletion of $CD20^+$ B cells in the peripheral blood with variable but often prolonged time to reconstitution and secondary drug-induced hypogammaglobulinaemia.[9] The kinetics of B-cell recovery in paediatric patients might not be the same as those described in adults given age-dependent development of the plasma cell compartment.[10] The effect of the addition of rituximab on immune function after completion of intensive lymphoma therapy in children and adolescents has not been well described. A prespecified secondary aim of the Inter-B-NHL-Ritux 2010 trial was to assess the effect of the addition of rituximab to conventional chemotherapy on laboratory correlates of immune function after completion of active treatment. Herein we present the results of this secondary analysis.

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Methods

Study design and participants

The primary study was led by two international paediatric oncology cooperative groups (the European Inter-group for Childhood Non-Hodgkin Lymphoma and the Children's Oncology Group) and took place in 176 centres in 12 countries (Australia, Belgium, Canada, France, Hong Kong, Hungary, Italy, the Netherlands, Poland, Spain, the UK, and the USA, appendix pp 3–7). It was an open-label, randomised, phase 3 trial of chemotherapy versus chemotherapy with rituximab. The chemotherapy regimen comprised an intensive paediatric chemotherapy backbone, modified from the therapy used in the FAB/LMB96 trial. Patients additionally treated with rituximab received the same backbone chemotherapy regimen with the addition of six doses of rituximab.[1] Details of the randomisation procedures have been reported previously.[1] After a planned interim analysis on Aug 14, 2015, showed superiority of the chemotherapy with rituximab group for event-free survival, the independent data and safety committee recommended stopping further randomisation. Following the results of the interim analysis a single-arm cohort of 120 patients were enrolled and non-randomly assigned to chemotherapy with rituximab in Europe and Hong Kong. Additionally, 33 randomly assigned patients who were still receiving chemotherapy after the closure of randomisation were recommended to receive rituximab regardless of the randomly assigned group (crossover part of the study). Eligible patients who were initially randomly assigned to receive chemotherapy with rituximab in this part of the study with immune data available, and eligible patients in the single-arm cohort with immune data available, were included in the immune study as an additional chemotherapy with rituximab group (figure).

Eligible patients were aged 6 months to 18 years with newly diagnosed, mature B-cell neoplasms (Burkitt lymphoma; diffuse large B-cell lymphoma; or high-grade, mature B-cell non-Hodgkin lymphoma, not otherwise specified) and St Jude stage III disease with a blood lactate dehydrogenase level more than twice the adult institutional upper limit of the normal range, or stage IV disease or leukaemia presentation. Patients with primary mediastinal (thymic) large B-cell lymphoma were not eligible.[1] Patients with known congenital immunodeficiency, HIV infection, or who were identified as hepatitis B carriers, and patients with previous exposure to rituximab were ineligible, as were patients who had received anticancer treatment except corticosteroids for less than 7 days duration in total. Patients who were pregnant or lactating were excluded on the basis of the toxicities of the chemotherapy regimen. There were no exclusions on the basis of performance status or laboratory-based measurements of organ function. Full eligibility criteria were published with the primary trial report.[1]

The study protocol was approved in each participating country by the relevant ethics and regulatory committees. Parents and patients (if appropriate for age) signed informed consent and assent forms before enrolment.

See Online for appendix

Procedures

The treatment protocol and specific chemotherapy regimens according to prognosis group (groups B, C1, and C3) have been published previously.[1] After prephase treatment with low-dose cyclophosphamide, vincristine, and prednisone, patients received four to six courses (according to disease stage) of intensive polychemotherapy. Rituximab was given as an intravenous infusion (375 mg/m²) 2 days before (day –2) and on the first day of each of two induction chemotherapy courses, and on the first day of each of two consolidation courses, for a total of six doses.

Baseline assessment of immune status (before treatment initiation) included total peripheral blood lymphocyte count and $CD19^+CD20^+$ B-cell enumeration by flow cytometry, measurement of serum IgG, IgA, and IgM concentrations, and measurement of serum antibodies to specific vaccines (polioviruses, tetanus, diphtheria toxoid, *Streptococcus pneumoniae* [pneumococcus], and *Haemophilus influenzae*). Peripheral blood count evaluation of other lymphocyte subsets ($CD3^+$, $CD3^+CD4^+$, and $CD3^+CD8^+$ T cells, as well as $CD3^-CD16^+$, $CD3^-CD56^+$, or $CD3^-CD16^+CD56^+$ natural killer [NK] cells) by flow cytometry was optional. These tests were done in the institutional clinical laboratories. Normative age-related cell count and immunoglobulin values were used according to reference standards (appendix pp 1–2).[11,12]

We defined additional cutoffs a priori on the basis of expert clinician input. For IgG and IgA, three levels for low concentrations (mildly low, moderately low, and severely low) were defined by the following thresholds: for IgG, if aged 10 years or younger, by the lower limit of the normal range (LLN) for age, 4 g/L, and 2 g/L, and if older than 10 years, by the LLN for age, 5 g/L, and 2 g/L; and for IgA, if aged 10 years or younger, by the LLN, 0·2 g/L, and 0·1 g/L, and if older than 10 years, by the LLN for age, 0·4 g/L, and 0·2 g/L. For IgM, two levels (mildly low and severely low) were defined by set thresholds: if aged 10 years or younger, by the LLN for age and 0·1 g/L, and if older than 10 years, by the LLN for age and 0·2 g/L. For $CD19^+CD20^+$ B cells, the absolute cell count threshold for mildly low was the LLN for age and for severely low was 100 cells per µL. For $CD3^+CD4^+$ T cells, the cell count threshold for mildly low was the LLN for age and for severely low was 200 cells per µL.

The same panel of immune assessments was collected at 1 month from the end of treatment (2 months after the start of the last course of chemotherapy) and at 1 year from the start of therapy. For patients with abnormal immunoglobulin or lymphocyte subset values at the 1-year timepoint, opportunistic follow-up was done

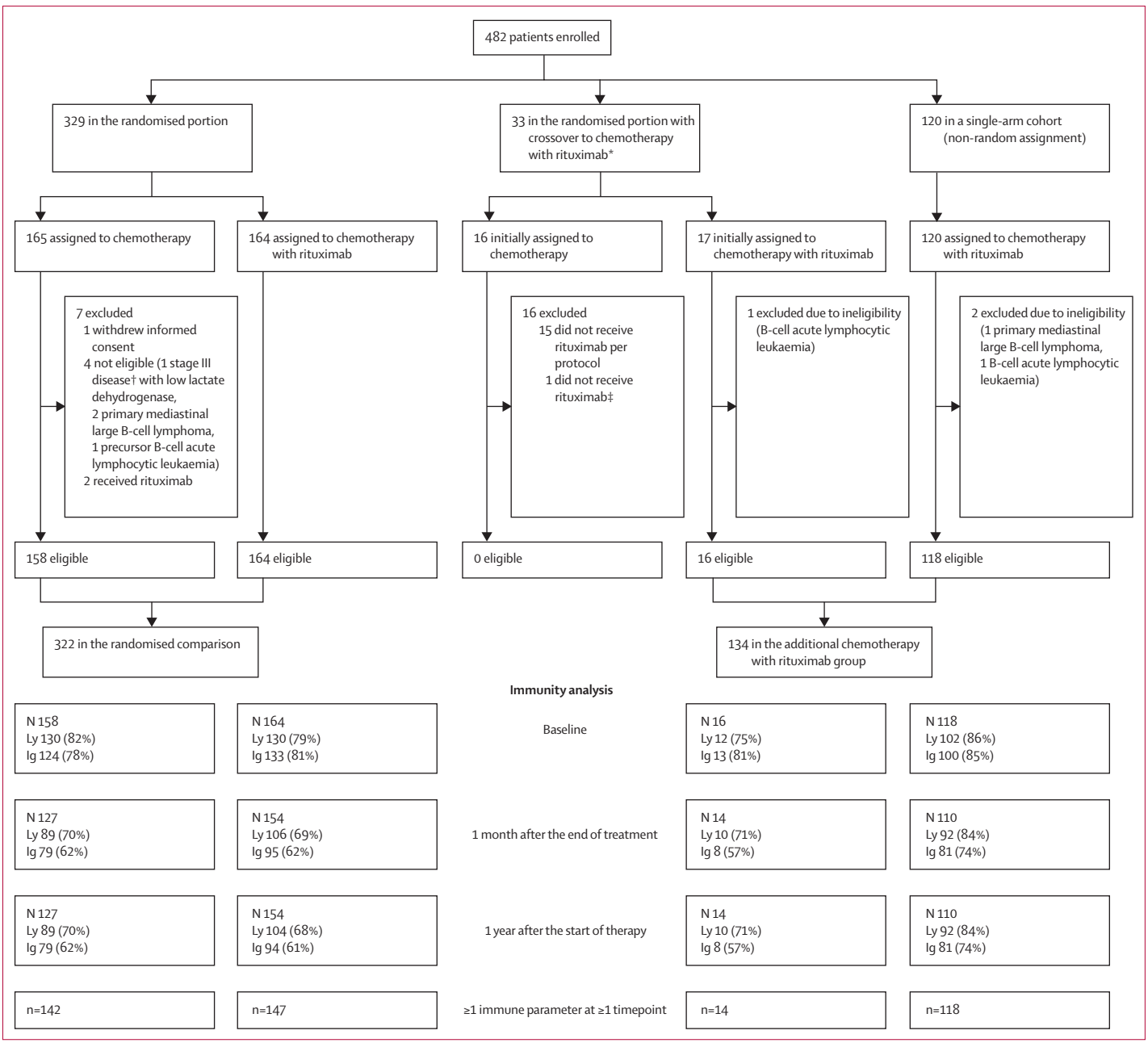

Figure: **Patient distribution for the secondary immunity analysis**
N=number of patients eligible for immunity analysis. Ly=number (%) of patients with available data on total lymphocyte counts. Ig=number (%) of patients with available data on IgG, IgA, or IgM. *Rituximab recommended after patients had started on study treatments. †St Jude staging system. ‡Not included in the randomised chemotherapy alone group to maintain comparability.

annually, within each subsequent year, up to 5 years from baseline or until both immunoglobulin concentrations and lymphocyte subsets normalised.

For serum antibodies to vaccine-preventable infections, samples were considered positive if the antibody titres were classified as being in the protective range by the respective clinical laboratory's reference specifications. The protocol did not prescribe revaccination, but suggested that live vaccines should not be administered until normalisation of B cell count, and that non-live vaccines could proceed according to institutional standards.

In addition, data were collected on immunoglobulin infusions and infections during therapy and follow-up. The protocol recommended that a clinically significant low concentration of immunoglobulins and the occurrence of severe or recurrent infections should be considered for decision making regarding use of immunoglobulin replacement therapy, in addition to

referring investigators to their respective national guidelines, if available.

All non-haematological toxicities of grade 3 or worse, according to the CTCAE (version 4), including infectious toxicities, were captured during each cycle of chemotherapy and in prespecified periods after therapy (6, 9, 12, 18, and 24 months from enrolment, then annually up to 5 years). Evaluations for autoimmune conditions were not a prespecified outcome and specific data were not collected. Among European patients only, according to study group regulations, for those with at least one clinically relevant low immunoglobulin concentration (ie, moderately or severely low) reported after therapy, additional data were collected for infectious events of any grade to identify patients who might have experienced clinically important but lower grade infectious adverse events. Additionally, the case report form was amended (May 16, 2014) to capture neutrophil counts during follow-up in European patients to assess post-treatment neutropenia, according to reference standards (appendix p 1).

Outcomes

The primary endpoint of the Inter-B-NHL Ritux 2010 study, event-free survival, has been reported previously.[1] Secondary endpoints were complete remission rate and overall survival between randomised arms; safety of therapy including grade 3 or worse non-haematological adverse events; and immune effects of therapy including the proportion of patients with low serum immunoglobulin concentrations and lymphocyte counts at 1 year after the start of therapy

Herein, we report the prespecified immune outcomes of lymphocyte enumeration (total and subset) and serum immunoglobulin concentrations at baseline, 1 month from the end of therapy, and 1 year after the start of treatment. These immune outcomes were chosen based on data in patients exposed to rituximab in other disease settings. Data beyond 1 year are also reported although interpretation is limited by small samples. The three main endpoints were total lymphocyte count, B-cell count, and IgG concentration on the basis of the established toxicity of rituximab. We also report on safety in terms of adverse events of grade 3 or worse severity during follow-up from 1 month after the end of therapy (toxicity during the treatment period has been reported previously[1]). Additional prespecified outcomes reported are the prevalence of serum antibodies to vaccine-preventable infections, and exposure to immunoglobulin replacement therapy and indications for use of this treatment.

Statistical analysis

Sample size was estimated for the primary study endpoint analysis of event-free survival.[1] At a one-sided 5% level of statistical significance, 72 events needed to be observed to have 90% power to detect a hazard ratio (HR) of events of 0·50, and an estimated 600 patients (300 per group) would need to be randomly assigned to observe this number of events. The study was stopped early after the first interim analysis, and only 362 patients were randomly assigned (181 in each group). To reach the 300 patients assigned to chemotherapy with rituximab, as initially planned to assess prespecified secondary endpoints in this population, the additional single-arm cohort of 120 patients who received chemotherapy with rituximab was enrolled (figure). The population assessed for immune endpoints was the eligible per-protocol population with at least one immune parameter at one timepoint, including the randomised and non-randomised groups. Comparisons of immune endpoints between the chemotherapy with rituximab arm and chemotherapy arm were restricted to the randomised part of the study (ie, before the planned interim analysis). Among the 33 patients in the crossover group, 15 initiated rituximab at variable points during therapy and received a range of total doses (figure) and these patients were not included in order to perform the analyses among patients with the same schedule of rituximab administration, as per the original trial protocol. The remaining patient in the crossover group did not receive rituximab, however was not included in the randomised chemotherapy alone group to maintain comparability, as their random assignment was after assignment of the first patient who received rituximab in the crossover group. Safety after the treatment period was assessed in the population eligible for the immunity study and followed up at least 3 months after the end of treatment and without cancer-related events. Vaccine serologies were studied in the population eligible for the immunity study without cancer-related events.

The data cutoff for this analysis was March 23, 2022, for patients enrolled by the European Inter-group for Childhood Non-Hodgkin Lymphoma, and Sept 30, 2019, for those enrolled by the Children's Oncology Group. Associations of study treatment with immune outcomes were assessed with use of univariable logistic regression (and Wald tests to derive p values), except when a group had events at 0% or 100%, in which case proportions were compared with Fisher's exact test. Odds ratios (ORs) for the chemotherapy with rituximab group versus the chemotherapy group were estimated in univariable logistic regression models. In addition, a descriptive analysis of all patients who received chemotherapy with rituximab (randomised and non-randomised groups) was done to improve estimation accuracy in these patients. Patients with bone marrow involvement with blast cells in peripheral blood at the time of diagnosis were removed from peripheral blood count analyses at baseline. Patients with cancer-related events, defined as relapse, progressive disease, second cancer, detection of residual viable tumour cells after the second consolidation course (ie, primary refractory disease), or death from any cause, were included only in the analyses of baseline data of cell counts and immunoglobulin concentrations. These patients were not included at later timepoints to avoid any effects of second-line chemotherapy on immunity.

As a post-hoc analysis, we used univariable and multivariable logistic regression to identify associations between initial patient and disease characteristics and IgG concentration at 1 year, in all patients treated with chemotherapy with rituximab. Additionally, analyses of lymphocytes and immunoglobulins were repeated post hoc with stratification by sex (not pre-specified in the protocol). A further post-hoc analysis was completed to describe the trajectory of abnormal measures of immune function in individual patients, to identify those who might be considered at risk of having an undiagnosed primary immunodeficiency.

In case of missing data on immune parameters (lymphocyte enumerations and serum immunoglobulins) and serologies, the analyses were done only in patients with available data. Data availability is presented in the appendix (pp 8–9). In the comparative randomised part of the study, a sensitivity analysis with multiple imputation was done for the three main endpoints (total lymphocyte count, B-cell count, and IgG concentration) at 1 month after the end of therapy and 1 year after the start of therapy.

The 5-year incidence of immunoglobulin infusion was estimated by the Kaplan-Meier method with 95% CIs estimated by the Rothman method. The HR for immunoglobulin infusion between the chemotherapy with rituximab and chemotherapy groups was estimated by Cox proportional hazards regression with 95% CIs based on the Wald test.

Analyses were done with SAS software (version 9.4). p values were two-sided at a significance level of 0·05. The Inter-B-NHL Ritux 2010 study is registered with ClinicalTrials.gov, NCT01516580.

Role of the funding source

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

Results

Between Dec 19, 2011, and June 13, 2017, 482 patients were enrolled at 176 participating centres (figure). 16 patients in the crossover portion of the study were excluded. Ten additional patients among the total enrolled cohort were not included in the present analysis: seven were deemed ineligible on the basis of histology or disease stage, two were in the chemotherapy alone group but received rituximab, and one withdrew consent. Additionally, 35 patients had no lymphocyte or immunoglobulin data submitted. Therefore, 421 patients were included in this analysis. In the randomised portion of the study (n=289), median follow-up was 58 months (IQR 43–65) and in the additional non-randomised chemotherapy with rituximab portion (n=132), median follow-up was 50 months (36–59).

Baseline characteristics of the 421 patients are presented in table 1. 344 (82%) boys and 77 (18%) girls were included, and mean age was 8·8 years (SD 4·1). Among patients in the randomised and non-randomised groups with available data, excluding those with bone marrow disease with peripheral blast cells at the time of diagnosis (n=84), 99 (34%) of 290 patients had lymphopenia at baseline. In patients with data on lymphocyte subsets, 24 (13%) of 188 had severely low $CD19^+CD20^+$ B cells (<100 cells per µL) and 19 (15%) of 125 had severely low $CD3^+CD4^+$ T cells (<200 cells per µL).

In the overall population, 178 (48%) of 368 with available data had serum IgG concentrations less than the LLN for age (table 1). Of these patients, a subset (n=12) had severely low IgG concentration (<2 g/L), including three (2%) of 124 in the randomised chemotherapy group, seven (5%) of 132 in the randomised chemotherapy with rituximab group, and two (2%) of 122 in the additional chemotherapy with rituximab cohort. In the overall study sample, a small number of patients had severely low serum concentrations of IgA (n=4) or IgM (n=7) at baseline. Blood cell counts and immunoglobulin concentrations at baseline according to bone marrow involvement and the presence of blast cells in blood are presented in the appendix (pp 10–11).

1 month after the end of therapy, patients in the randomised chemotherapy with rituximab group were significantly more likely than those in the chemotherapy group to have lymphopenia (86 [81%] of 106 *vs* 53 [60%] of 89, OR 2·92 [95% CI 1·53–5·57], p=0·0011) and B-cell lymphopenia (covering both mildly low and severely low $CD19^+CD20^+$ B cells; 72 [96%] of 75 *vs* 36 [64%] of 56, OR 13·33 [3·71–47·84], p<0·0001; table 2). 1 year after the start of therapy, these differences between the two groups were no longer apparent. The proportions of patients with low T cells or NK cells were not significantly different between the randomised groups at either timepoint. Among all patients who received rituximab, the proportions of patients with low cell counts were similar in the randomised group and the additional non-randomised group (appendix pp 17–18). Of note, no patients in the randomised and non-randomised chemotherapy with rituximab groups were identified as having severe neutropenia at the follow-up timepoints.

1 month after the end of therapy and persisting 1 year after the start of therapy, patients in the randomised chemotherapy with rituximab group were significantly more likely to have low IgG, IgA, and IgM serum concentrations than patients in the chemotherapy group (table 2). Low IgG (hypogammaglobulinemia) was present in 67 (71%) of 95 patients who received chemotherapy with rituximab versus 37 (47%) of 79 who received chemotherapy (OR 2·72 [95% CI 1·45–5·07], p=0·0017) 1 month after the end of therapy; and 52 (55%) of 94 versus 16 (25%) of 63 (OR 3·64 [1·81–7·31], p=0·0003) 1 year after the start of therapy. No patients (0 of 79) in the chemotherapy group had severely low IgG (<2 g/L) at the 1-month or 1-year timepoints. Conversely, among all patients who received rituximab in the randomised and

	Randomised chemotherapy group (n=142)	Randomised chemotherapy with rituximab group (n=147)	Additional chemotherapy with rituximab group (n=132)*	Chemotherapy with rituximab: all patients (n=279)†	Total patients (n=421)
Patient and disease features					
Sex					
Male	120 (85%)	122 (83%)	102 (77%)	224 (80%)	344 (82%)
Female	22 (15%)	25 (17%)	30 (23%)	55 (20%)	77 (18%)
Age, years					
Mean (SD; range)	8·4 (4·3; 2–17)	9·1 (4·0; 2–17)	8·9 (4·1; 2–17)	9·0 (4·0; 2–17)	8·8 (4·1; 2–17)
<6	51 (36%)	40 (27%)	39 (30%)	79 (28%)	130 (31%)
6 to <12	59 (42%)	67 (46%)	59 (45%)	126 (45%)	185 (44%)
≥12	32 (23%)	40 (27%)	34 (26%)	74 (27%)	106 (25%)
Pathological diagnosis					
Burkitt lymphoma	125 (88%)	127 (86%)	121 (92%)	248 (89%)	373 (89%)
Diffuse large B-cell lymphoma	12 (8%)	17 (12%)	10 (8%)	27 (10%)	39 (9%)
High-grade mature B-cell lymphoma, not otherwise specified	5 (4%)	3 (2%)	1 (1%)	4 (1%)	9 (2%)
Prognosis group					
Group B high risk	74 (52%)	72 (49%)	61 (46%)	133 (48%)	207 (49%)
Group C without cerebrospinal fluid blast cells (C1)	55 (39%)	60 (41%)	53 (40%)	113 (41%)	168 (40%)
Group C with cerebrospinal fluid blast cells (C3)	13 (9%)	15 (10%)	18 (14%)	33 (12%)	46 (11%)
St Jude stage					
Stage III	66 (46%)	64 (44%)	53 (40%)	117 (42%)	183 (43%)
Stage IV	28 (20%)	28 (19%)	35 (27%)	63 (23%)	91 (22%)
Leukaemia presentation (mature B-cell acute leukaemia)	48 (34%)	55 (37%)	44 (33%)	99 (35%)	147 (35%)
Bone marrow involvement					
No	79 (56%)	81 (55%)	70 (53%)	151 (54%)	230 (55%)
Yes: blast cells <25%	17 (12%)	12 (8%)	18 (14%)	30 (11%)	47 (11%)
Yes: blast cells ≥25%	46 (32%)	54 (37%)	44 (33%)	98 (35%)	144 (34%)
CNS involvement					
No	104 (73%)	106 (72%)	90 (68%)	196 (70%)	300 (71%)
Yes	38 (27%)	41 (28%)	42 (32%)	83 (30%)	121 (29%)
Lactate dehydrogenase concentration					
≤2 times the ULN	17 (12%)	15 (10%)	20 (15%)	35 (13%)	52 (12%)
>2 times the ULN	125 (88%)	132 (90%)	112 (85%)	244 (87%)	369 (88%)
Lymphocyte values‡					
Total lymphocytes	n=104	n=105	n=81	n=186	n=290
Low	33 (32%)	37 (35%)	29 (36%)	66 (35%)	99 (34%)
$CD19^+CD20^+$ B cells	n=73	n=71	n=44	n=115	n=188
Mildly low	16 (22%)	21 (30%)	6 (14%)	27 (23%)	43 (23%)
Severely low	11 (15%)	9 (13%)	4 (9%)	13 (11%)	24 (13%)
$CD3^+$ T cells	n=49	n=57	n=38	n=95	n=144
Low	31 (63%)	37 (65%)	27 (71%)	64 (67%)	95 (66%)
$CD3^+CD4^+$ T cells	n=40	n=49	n=36	n=85	n=125
Mildly low	18 (45%)	21 (43%)	17 (47%)	38 (45%)	56 (45%)
Severely low	6 (15%)	8 (16%)	5 (14%)	13 (15%)	19 (15%)
$CD3^+CD8^+$ T cells	n=40	n=49	n=36	n=85	n=125
Low	26 (65%)	34 (69%)	20 (56%)	54 (64%)	80 (64%)
NK cells§	n=32	n=38	n=35	n=73	n=105
Low	20 (62%)	19 (50%)	17 (49%)	36 (49%)	56 (53%)

(Table 1 continues on next page)

	Randomised chemotherapy group (n=142)	Randomised chemotherapy with rituximab group (n=147)	Additional chemotherapy with rituximab group (n=132)*	Chemotherapy with rituximab: all patients (n=279)†	Total patients (n=421)
(Continued from previous page)					
Immunoglobulin values					
IgG	n=124	n=132	n=112	n=244	n=368
Mildly low	32 (26%)	30 (23%)	35 (31%)	65 (27%)	97 (26%)
Moderately low	21 (17%)	29 (22%)	19 (17%)	48 (20%)	69 (19%)
Severely low	3 (2%)	7 (5%)	2 (2%)	9 (4%)	12 (3%)
IgA	n=121	n=133	n=110	n=243	n=364
Mildly low	5 (4%)	15 (11%)	6 (5%)	21 (9%)	26 (7%)
Moderately low	6 (5%)	3 (2%)	5 (5%)	8 (3%)	14 (4%)
Severely low	0 (0%)	2 (2%)	2 (2%)	4 (2%)	4 (1%)
IgM	n=123	n=133	n=111	n=244	n=367
Mildly low	10 (8%)	18 (14%)	19 (17%)	37 (15%)	47 (13%)
Severely low	1 (1%)	5 (4%)	1 (1%)	6 (2%)	7 (2%)

Data are n (%) unless otherwise specified; n is given if measures were not available for all individuals in the group. Submission of data for prespecified non-optional measures for the secondary analysis (total lymphocytes, $CD19^+CD20^+$ B cells, and immunoglobulins) was not optimally complied with. Ethnicity and race data are not provided as in Europe the standard rule for clinical trials does not allow for collection of these data unless authorisation is requested and granted. Reference ranges of blood cell counts and immunoglobulin concentrations are provided in the appendix (pp 1-2). NK=natural killer. LLN=lower limit of the normal range. ULN=upper limit of the normal range. *The non-comparative chemotherapy with rituximab group of patients from the crossover and single-arm components of the study (figure). †Patients from the randomised chemotherapy with rituximab group and the non-comparative chemotherapy with rituximab groups. ‡Excluding patients with bone marrow disease with peripheral blast cells at the time of diagnosis for lymphocyte counts. §NK cells identified as $CD3^-CD16^+$, $CD3^-CD56^+$, or $CD3^-CD16^+CD56^+$.

Table 1: **Patient clinical and laboratory characteristics at baseline**

non-randomised groups, severely low IgG was reported in eight of 184 patients (4%) at the 1-month timepoint and three of 174 patients (2%) at the 1-year timepoint.

Among patients in the randomised groups, a sensitivity analysis with multiple imputation of missing data for total lymphocytes, B-cells, and IgG showed a reduced effect of rituximab on these three parameters compared with the crude analysis of observed cases, although the differences between groups were still significant (appendix p 12). When the main analysis was stratified by sex, significant differences remained in boys for total lymphocytes, B cells, and immunoglobulins 1 month after the end of therapy and for immunoglobulins 1 year after the start of therapy (appendix pp 13–14). Due to the small number of girls (between 15 and 30 depending on the immune parameter), the power of the analyses was low and no conclusion can be drawn from the tests comparing the treatment groups (appendix pp 15–16).

Beyond the 1-year timepoint, analysis was limited by small numbers of patients with available data. At the 2-year follow-up, one (7%) of 15 patients who received chemotherapy and one (3%) of 38 patients who received chemotherapy with rituximab (randomised and non-randomised groups) had severely low $CD19^+CD20^+$ B cells. At this timepoint, no patients (out of 27 with available data) who received chemotherapy had moderately or severely low IgG concentration; whereas, nine (11%) of 81 patients who received chemotherapy with rituximab had moderately low (n=7) or severely low (n=2) IgG concentrations.

Of all patients who received chemotherapy with rituximab, age younger than 15 years at baseline, receipt of group C versus group B therapy, and low IgG concentration at baseline were significantly associated with low IgG concentration at 1 year after the start of therapy in multivariable analyses (table 3).

During therapy and in post-therapy follow-up, a greater number of patients in the randomised chemotherapy with rituximab group received one or more immunoglobulin infusions than in the chemotherapy group (26 of 164 *vs* nine of 158, 5-year incidence rate 16% [95% CI 11–22] *vs* 7% [3–12], hazard ratio [HR] 2·63 (95% CI 1·23–5·62; p=0·010; appendix pp 19–20). The median number of doses per patient was three (IQR 2–7; range 1–50) in the chemotherapy group and two (1–3; 1–18) in the chemotherapy with rituximab group. The most common indication reported was hypogammaglobulinemia alone, followed by frequent infections with or without documented hypogammaglobulinemia. There was geographical variability in the use of immunoglobulin replacement. Among patients in the randomised and non-randomised chemotherapy with rituximab groups, the 5-year incidence of Ig infusions was 16% (95% CI 12–20; 47 of 298 patients; appendix p 19), ranging from 0% (0 of 29) to 29% (16–47; nine of 31) in countries that enrolled at least ten patients (data not shown).

Toxicity during the treatment period has been reported previously.[1] Among the 279 patients who received rituximab in either the randomised or non-randomised portions of the study, 16 (6%) did not receive the planned

	1 month after the end of chemotherapy				1 year after the start of therapy			
	Randomised chemotherapy group	Randomised chemotherapy with rituximab group	OR (95%CI)*, p value†	Chemotherapy with rituximab: all patients‡	Randomised chemotherapy group	Randomised chemotherapy with rituximab group	OR (95%CI)*, p value†	Chemotherapy with rituximab: all patients‡
Lymphocytes								
Total lymphocytes	n=89	n=106	..	n=208	n=81	n=104	..	n=195
Low	53 (60%)	86 (81%)	2·92 (1·53–5·57), p=0·0011	168 (81%)	13 (16%)	18 (17%)	1·09 (0·50–2·39), p=0·82	33 (17%)
$CD19^+CD20^+$ B cells	n=56	n=75	..	n=138	n=47	n=67	..	n=124
Mildly low	5 (9%)	3 (4%)	..	7 (5%)	3 (6%)	10 (15%)	..	18 (15%)
Severely low	31 (55%)	69 (92%)	64% *vs* 96%; 13·33 (3·71–47·84), p<0·0001	126 (91%)	6 (13%)	8 (12%)	19% *vs* 27%; 1·55 (0·63–3·84), p=0·34	13 (10%)
$CD3^+$ T cells	n=41	n=67	..	n=135	n=40	n=61	..	n=120
Low	36 (88%)	63 (94%)	2·19 (0·55–8·67), p=0·27	124 (92%)	14 (35%)	25 (41%)	1·29 (0·56–2·95), p=0·55	49 (41%)
$CD3^+CD4^+$ T cells	n=38	n=64	..	n=124	n=37	n=58	..	n=110
Mildly low	23 (61%)	34 (53%)	..	62 (50%)	14 (38%)	24 (41%)	..	48 (44%)
Severely low	15 (39%)	28 (44%)	100% *vs* 97%; not estimable, p=0·53	58 (47%)	3 (8%)	4 (7%)	46% vs 48%; 1·10 (0·48–2·51), p=0·82	4 (4%)
$CD3^+CD8^+$ T cells	n=37	n=63	..	n=123	n=37	n=58	..	n=110
Low	22 (59%)	47 (75%)	2·00 (0·84–4·77), p=0·12	89 (72%)	10 (27%)	18 (31%)	1·22 (0·49–3·03), p=0·68	33 (30%)
NK cells	n=32	n=51	..	n=106	n=33	n=51	..	n=99
Low	12 (37%)	19 (37%)	0·99 (0·40–2·47), p=0·98	39 (37%)	8 (24%)	5 (10%)	0·34 (0·10–1·15), p=0·082	12 (12%)
Immunoglobulins								
IgG	n=79	n=95	..	n=184	n=63	n=94	..	n=174
Mildly low	23 (29%)	33 (35%)	..	65 (35%)	12 (19%)	32 (24%)	..	64 (37%)
Moderately low	14 (18%)	30 (32%)	..	56 (30%)	4 (6%)	18 (19%)	..	34 (20%)
Severely low	0	4 (4%)	47% *vs* 71%; 2·72 (1·45–5·07), p=0·0017	8 (4%)	0	2 (2%)	25% *vs* 55%; 3·64 (1·81–7·31), p=0·0003	3 (2%)
IgA	n=78	n=95	..	n=181	n=64	n=92	..	n=170
Mildly low	9 (12%)	21 (22%)	..	47 (26%)	2 (3%)	11 (12%)	..	20 (12%)
Moderately low	3 (4%)	8 (8%)	..	13 (7%)	0	5 (5%)	..	8 (5%)
Severely low	0	3 (3%)	15% *vs* 34%; 2·79 (1·32–5·90), p=0·0071	4 (2%)	1 (2%)	0	5% *vs* 17%; 4·28 (1·19–15·37), p=0·026	1 (1%)
IgM	n=78	n=95	..	n=180	n=64	n=92	..	n=170
Mildly low	30 (38%)	52 (55%)	..	102 (57%)	4 (6%)	21 (23%)	..	35 (21%)
Severely low	4 (5%)	16 (17%)	44% *vs* 72%; 3·26 (1·73–6·13), p=0·0002	26 (14%)	1 (2%)	0	8% *vs* 23%; 3·49 (1·24–9·82), p=0·018	1 (1%)

n is the number of individuals with data on each measure. Reference ranges of blood cell counts and immunoglobulin concentrations are provided in the appendix (pp 1–2). NK=natural killer cells. OR=odds ratio. *ORs from univariable logistic regression are for the randomised chemotherapy with rituximab group versus the randomised chemotherapy group, including all patients with values below the lower limit of the normal range (ie, patients in the "low" category for total lymphocytes, CD3+ T cells, CD3+CD8+ T cells, and NK cells; patients in the "mildly low" and "severely low" categories for CD19+CD20+ B cells, CD3+CD4+ T cells, and IgM; and patients in the "mildly low", "moderately low", and "severely low" categories for IgG and IgA); the compared percentages are given when data from more than one category were combined. †p values are for the comparison of all patients with measures below the LLN between the randomised groups and are from Wald tests for the logistic regression, except for CD3+CD4+ T cells at 1 month for which the p value is from Fisher's exact test. ‡Patients from the randomised chemotherapy with rituximab group and the additional (non-comparative) chemotherapy with rituximab group (figure).

Table 2: **Lymphocytes and immunoglobulins at the 1-month and 1-year timepoints**

six doses (two patients received one dose, three patients received two doses, six patients received four doses, and five patients received five doses), with toxicity being identified as the reason for not completing the planned number of infusions in nine (56%) patients. Among the 279 patients who received chemotherapy with rituximab, there were four (1%) treatment-related deaths during therapy, and among the 142 patients in the chemotherapy group, there were two (1%) treatment-related deaths during therapy. After the 1-month post-therapy

timepoint, there were no deaths in patients who remained in remission. In the follow-up period starting 1 month from the end of therapy, grade 3 or worse non-haematological adverse events were rare (table 4). One patient in the randomised chemotherapy with rituximab group with severely low IgG at 1-month post-therapy had a grade 4 (life-threatening) infectious event of polymicrobial bacterial sepsis, 2 months after the final chemotherapy administration. Additionally, five patients, all treated with rituximab, including four in the randomised group, had grade 3 infections between 1 and 29 months after completion of therapy, including Epstein-Barr virus infection (n=1), herpes zoster virus infection (n=1), bacterial infection (n=1), and pneumonia (n=2). Among these six patients with infectious complications of grade 3 or worse after therapy, two patients received immunoglobulin replacement after the infectious event. When assessing immunoglobulin concentrations and lower grade infectious events in the European cohort, 43 patients were identified as having low IgG in the follow-up period, five of whom also had lower grade (CTACE grade 1–2) infectious events, including sinusitis, lung infection, impetigo, pharyngitis, and viral infection (in one patient each). All of these patients were in the chemotherapy with rituximab groups.

At baseline, in all patients with available serology data, protective serologies were identified in 74 (59%) of

	Univariable analysis				Final multivariable model*			
	Normal IgG concentration (n=73)	IgG concentration less than the LLN (n=101)	OR (95% CI)	p value†	Normal IgG concentration (n=60)	IgG concentration less than the LLN (n=95)	Adjusted OR (95% CI)	p value†
Sex								
Male	61 (43%)	80 (57%)	1 (ref)	..	NA	NA	..	..
Female	12 (36%)	21 (64%)	1·33 (0·61–2·92)	0·47	NA	NA	..	..
Age at baseline, years								
Mean (SD)	9·5 (4·5)	8·1 (3·6)	NA	..	9·2 (4·5)	8·0 (3·7)	NA	NA
<15‡	57 (37%)	97 (63%)	6·81 (2·17–21·36)	..	48 (35%)	91 (65%)	9·13 (2·39–34·80)	..
≥15‡	16 (80%)	4 (20%)	1 (ref)	0·0010	12 (75%)	4 (25%)	1 (ref)	0·0012
Pathological diagnosis								
Burkitt lymphoma	66 (42%)	93 (58%)	1 (ref)	..	NA	NA	..	..
Diffuse large B-cell lymphoma	7 (54%)	6 (46%)	0·61 (0·20–1·89)	0·39§	NA	NA	..	..
Other	0 (0%)	2 (100%)	Not estimable	..	NA	NA	..	..
Prognosis group								
Group B	36 (51%)	35 (49%)	1 (ref)	..	30 (48%)	32 (52%)	1 (ref)	..
Group C1	31 (38%)	51 (62%)	1·69 (0·89–3·22)	..	26 (35%)	48 (65%)	3·48 (1·51–8·06)	..
Group C3	6 (29%)	15 (71%)	2·57 (0·89–7·38)	0·12	4 (21%)	15 (79%)	3·08 (0·87–10·92)	0·0087
St Jude stage								
Stage III	30 (48%)	33 (52%)	1 (ref)	..	NA	NA	..	..
Stage IV	18 (46%)	21 (54%)	1·06 (0·47–2·36)	..	NA	NA	..	..
Leukaemia presentation (mature B-cell acute leukaemia)	25 (35%)	47 (65%)	1·71 (0·85–3·42)	0·27	NA	NA	..	..
Bone marrow involvement								
Blast cells <25%	48 (47%)	54 (53%)	1 (ref)	..	NA	NA	..	..
Blast cells ≥25%	25 (35%)	47 (65%)	1·67 (0·90–3·11)	0·11	NA	NA	..	..
CNS involvement								
No	54 (45%)	67 (55%)	1 (ref)	..	NA	NA	..	..
Yes	19 (36%)	34 (64%)	1·44 (0·74–2·81)	0·28	NA	NA	..	..
Lactate dehydrogenase concentration at baseline								
≤2 times the ULN	12 (55%)	10 (45%)	1 (ref)	..	11 (55%)	9 (45%)	1 (ref)	..
>2 times the ULN	61 (40%)	91 (60%)	1·79 (0·73–4·40)	0·20	49 (36%)	86 (64%)	2·52 (0·86–7·40)	0·091
IgG concentration at baseline								
At or greater than the LLN	39 (48%)	42 (52%)	1 (ref)	..	39 (48%)	42 (52%)	1 (ref)	..
Less than the LLN	21 (28%)	53 (72%)	2·34 (1·20–4·57)	0·012	21 (28%)	53 (72%)	2·51 (1·18–5·34)	0·017

Odds ratios are for the odds of IgG concentration at 1 year being less than the LLN. NA=not applicable (not tested). LLN=lower limit of the normal range. ULN=upper limit of the normal range. *Model based on 155 patients due to missing data on IgG concentration at baseline for 19 patients; only variables with p<0·10 in the initial multivariable model (not shown) were retained in the final multivariable model (backward selection). †Wald test p values. ‡US National Cancer Institute threshold to separate children and adolescents. §Burkitt lymphoma versus diffuse large B-cell lymphoma.

Table 3: **Patient and disease features associated with low IgG concentration at 1 year after the start of therapy in patients treated with chemotherapy with rituximab**

126 patients for poliovirus, 162 (74%) of 220 for tetanus, 128 (70%) of 182 for diphtheria, 88 (63%) of 139 for pneumococcus, and 72 (56%) of 129 for *H influenzae* (appendix p 21). Vaccine serology data were obtained on small numbers of patients at the follow-up timepoints. Across the treatment groups, some patients with documented positive titres at baseline had negative titres after completion of therapy, including four (9%) of 47 patients for polio, 24 (24%) of 99 for tetanus, 18 (23%) of 79 for diphtheria, 21 (42%) of 50 for pneumococcus, and 14 (35%) of 40 for *H influenzae* (appendix p 22). Conversely, a small number of patients with negative serologies, either at baseline or after therapy, were documented to have protective antibodies after vaccinations administered during the study, although several of these patients had received intercurrent immunoglobulin replacement therapy (appendix p 23).

Although patients with known immunodeficiency were excluded from the trial, B-cell non-Hodgkin lymphoma can be the presenting feature of primary immunodeficiency. We therefore sought to understand individual immune trajectories with a view to identifying patients with increased risk of underlying immunodeficiency. 24 (13%) of 188 patients had severely low $CD19^+CD20^+$ B cells (<100 cells µL) at baseline (table 1). Three of these patients continued to have severely low B cells at the last timepoint evaluated, two at 1 year after the start of therapy (one each in the randomised chemotherapy with rituximab group and chemotherapy group) and one at the 2-year follow-up (chemotherapy group). Similarly, 19 (15%) of 125 patients had severely low $CD3^+CD4^+$ T cells (<200 cells per µL) at baseline. Three of the 19 patients with severely low $CD3^+CD4^+$ T cells also had severely low $CD19^+CD20^+$ B cells at baseline. Three patients continued to have severely low $CD3^+CD4^+$ T cells at the last timepoint evaluated, at 7 months (opportunistic follow-up), 1 year, and approximately 3 years after the start of therapy. One of these patients also had severely low $CD19^+CD20^+$ B cells at the last timepoint evaluated.

12 (3%) of 368 patients had severely low concentrations of IgG (<2 g/L) at baseline (table 1), five of whom had concurrent severely low $CD19^+CD20^+$ B cells. None of these patients had severely low IgG at the 1-year timepoint. Conversely, three patients had severely low IgG concentrations at the 1-year timepoint, all in the chemotherapy with rituximab group (table 2). All three of these patients had IgG concentrations in the normal range at the time of enrolment.

In total, 47 (12%) of 378 patients had severely low $CD19^+CD20^+$ B cells or $CD3^+CD4^+$ T cells, or severely low IgG concentration, at baseline. Follow-up on immune parameters was available for 24 of these patients. Among these patients, 18 (75%) had normal immune parameters at their last evaluation (1 year from the start of therapy) and six (25%) continued to have abnormal immune tests at their last evaluation at or after 1-year timepoint. Only one of these patients was identified as having an acute infection during follow-up, which was the episode of grade 4 polymicrobial bacterial sepsis.

	Randomised chemotherapy group (n=127)	Randomised chemotherapy with rituximab group (n=154)	Additional chemotherapy with rituximab group (n=124)
Infection grade ≥3	0	5	1
Epstein Barr virus infection			
Grade 3	0	1	0
Herpes zoster virus infection			
Grade 3	0	0	1
Bacterial infection			
Grade 3	0	3*	0
Grade 4	0	1	0
Neurotoxicity grade ≥3	0	0	2
Tremor			
Grade 3	0	0	1
Peripheral sensory neuropathy			
Grade 3	0	0	1

Numbers of patients with each event are shown. *Including two patients with pneumonia.

Table 4: **Non-haematological adverse events of grade 3 or worse severity during follow-up**

Discussion

This study showed that differences in B-cell lymphopenia, including severe B-cell lymphopenia, between the chemotherapy with rituximab and chemotherapy groups resolved by 1 year after the start of therapy; whereas, patients receiving chemotherapy with rituximab were more likely to have persistently low immunoglobulin concentrations. Compliance with follow-up data submission after the 1-year timepoint was suboptimal, making extended analyses limited and based on small numbers.

Rituximab depletes $CD20^+$ B cells.[9] All B-lineage haematopoietic cells express CD20 except the earliest precursor cells, pro-B cells, and terminally differentiated antibody-producing plasma cells.[13] Exposure to rituximab causes predictable transient B-cell depletion in blood and, although plasma cells are not directly affected by the $CD20^+$ B-cell depletion, such exposure is associated with prolonged hypogammaglobulinemia in some patients. In a large cohort study of adult patients treated with rituximab for various indications, of those who had normal immunoglobulin concentrations at baseline, 66 (19%) of 342 had mild to severe deficiency 18 months after rituximab exposure.[14] Similar results have been described in other patient groups.[15] Rituximab-related hypogammaglobulinemia is more common in children than adults, which is potentially related to age-dependent development of the immune system and a lower proportion of memory B-cells and plasma cells in children.[16] In our study, 52 (55%) of 94 patients who received rituximab and chemotherapy (randomised group) and were tested at

1 year after the start of therapy had low IgG concentrations, compared with 16 (25%) of 63 of those who received chemotherapy alone. In patients exposed to rituximab across the study, younger age, worse prognostic group, and low IgG concentration at baseline were predictive of low IgG at 1-year after the start of therapy.

Hypogammaglobulinemia is associated with risk of bacterial infections. Additionally, exposure to rituximab has been associated with rare cases, primarily in adult patients, of progressive multifocal leukoencephalopathy, chronic enteric viral infections, and viral reactivation syndromes, most notably hepatitis B.[17,18] In this study we observed no fatal infections in the follow-up period, however a small number of patients had severe infections, all of whom had received rituximab. Severe infections in the post-therapy period are expected to be rare events, and therefore registry data capturing large patient numbers might be required to accurately quantify this risk.

General guidance for revaccination in children after cancer therapy is variable, with some guidelines suggesting revaccination starting at 6 months.[19,20] Data to inform vaccination after rituximab exposure are scarce. In adult patients, previous exposure to rituximab is a risk factor for poor vaccine response to a number of vaccines including for influenza, tetanus, *H influenzae*, pneumococcus, and SARS-Cov-2, with variability in antibody response based on timing related to rituximab administration, and with data suggesting that patients might require repeated vaccinations.[21–24] The data available on loss of vaccine-protective antibodies in this study is limited by the small sample, however many patients with documented protective antibodies before therapy had loss of protective titres during follow-up. Identifying the utility of post-treatment serological testing, or other measures of immunity, and effectiveness of revaccination, represents a substantial research gap within paediatric oncology. These data will be crucially important in informing national organisations on the development and optimisation of guidance for the immunisation of children after immune therapies for cancer, including rituximab.

Primary immunodeficiency is a known risk factor for the development of lymphomas in children.[25,26] The true prevalence of primary immunodeficiency at the point of lymphoma diagnosis is unknown.[27] Although pre-existing immunodeficiency was an exclusion criterion in the Inter-B-NHL-Ritux 2010 trial, it is likely that a small number of patients in our study had undiagnosed primary immunodeficiency. When assessing immune trajectories for a subset of patients in this study, most children with markedly abnormal lymphocytes or immunoglobulins at baseline had normalisation of those parameters after treatment; thus immunological investigations at the time of lymphoma diagnosis do not appear to offer a specific approach to screening for primary immunodeficiency. Conversely, in a previous study, children with prolonged hypogammaglobulinemia after rituximab therapy for autoimmune cytopenias were more likely to have primary immunodeficiency diagnosed than those who had recovery of immunoglobulin concentrations within 12 months of completing therapy.[28] In our study, around 2% of children (nine of 378) had persisting hypogammaglobulinemia or lymphopenia 1 year after therapy. Further investigation of children experiencing prolonged hypogammaglobulinemia or lymphopenia after treatment for lymphoma might help to identify those with an immunological predisposition. Additionally, the increasing use of either targeted or genome-wide sequencing approaches is likely to identify children with previously undiagnosed primary immunodeficiency in a more precise and timely manner and might inform treatment approaches.

This study has several limitations. First, in the follow-up period only infectious events meeting the criteria for a severe (grade 3 or worse) adverse event required reporting. The incidence and nature of lower grade infectious toxicities, such as those treated as an outpatient, were captured in only a subset of patients identified as having hypogammaglobulinemia. Second, laboratory evaluations were not completed at a centralised facility, which might have led to some variability in reporting. Third, not all laboratory evaluations were completed on all patients at all timepoints, and thus data are available only on a subset of patients and are primarily limited to two timepoints (1 month after the end of therapy and 1 year after the start of therapy). For immunoglobulins, at the 1-year monitoring point, physicians checked IgG concentration more frequently in patients treated with rituximab than in those treated without rituximab, and in patients assigned to prognostic group C versus prognostic group B (data not shown). This differential IgG monitoring in the groups with and without rituximab could create a bias in the estimation of the effect of rituximab on IgG. However, as IgG assessment was done less frequently in patients who had not received rituximab, we can assume that, if there was a bias, it would have been more likely to lead to an overestimation of the negative effect of rituximab on IgG, and thus our results would be conservative and cautious. Fourth, vaccination records before diagnosis were not available to inform interpretation of baseline vaccine titres. Fifth, the laboratory studies included were quantitative measures and not functional immunological studies, and therefore only describe the quantitative and not qualitative measures of immune function. Finally, the potential confounding effect of immunoglobulin replacement therapy on serological evaluation for vaccine-preventable infections was not accounted for in the analysis.

In summary, the addition of rituximab had measurable effects on laboratory measures of immune function after therapy in children receiving intensive therapy for mature B-cell lymphoma. Notably, rituximab was associated with risk of prolonged hypogammaglobulinemia, warranting monitoring during the post-therapy period. Methods for the identification of children and adolescents who are most likely to benefit from immunoglobulin replacement therapy and revaccination is a substantial research gap, as

is a structured approach to the identification of patients with possible underlying primary immunodeficiencies.

Contributors

CP, TGG, AA, and VM-C designed the study. All authors contributed to data collection. SA, AA, SB, and VM-C analysed data, and accessed and verified the underlying data. All authors contributed to the drafting, review, and revision of this manuscript, and vouch for adherence of the study to the protocol and the accuracy of the results. 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

GAAB provides consultancy to Roche, Novartis, Merck, and Janssen. AA, CP, and VM-C received grant support for the present study, paid to their institution, from F Hoffmann–La Roche. All other authors declare no competing interests.

Data sharing

De-identified individual participant data collected during the Inter-B-NHL Ritux 2010 trial will be made available for the purposes of meta-analysis. Proposals should be submitted to Gustave Roussy (https://redcap.link/DataRequestClinicalTrialsGustaveRoussy). A document indicating the objective of the research, the methodology, the statistical analysis plan, and the variables within the trial database required for the research must be submitted. A scientific board at Gustave Roussy will review and approve the request. A specific agreement between the sponsor and the researcher is requested for data transfer. This data transfer agreement details both parts' responsibilities to ensure the required level of data integrity and legal and ethical obligations.

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