ORIGINAL ARTICLE

Rituximab in B-Lineage Adult Acute Lymphoblastic Leukemia

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ABSTRACT

BACKGROUND

Treatment with rituximab has improved the outcome for patients with non-Hodgkin's lymphoma. Patients with B-lineage acute lymphoblastic leukemia (ALL) may also have the CD20 antigen, which is targeted by rituximab. Although single-group studies suggest that adding rituximab to chemotherapy could improve the outcome in such patients, this hypothesis has not been tested in a randomized trial.

METHODS

We randomly assigned adults (18 to 59 years of age) with CD20-positive, Philadelphia chromosome (Ph)–negative ALL to receive chemotherapy with or without rituximab, with event-free survival as the primary end point. Rituximab was given during all treatment phases, for a total of 16 to 18 infusions.

RESULTS

From May 2006 through April 2014, a total of 209 patients were enrolled: 105 in the rituximab group and 104 in the control group. After a median follow-up of 30 months, event-free survival was longer in the rituximab group than in the control group (hazard ratio, 0.66; 95% confidence interval [CI], 0.45 to 0.98; P=0.04); the estimated 2-year event-free survival rates were 65% (95% CI, 56 to 75) and 52% (95% CI, 43 to 63), respectively. Treatment with rituximab remained associated with longer event-free survival in a multivariate analysis. The overall incidence rate of severe adverse events did not differ significantly between the two groups, but fewer allergic reactions to asparaginase were observed in the rituximab group.

CONCLUSIONS

Adding rituximab to the ALL chemotherapy protocol improved the outcome for younger adults with CD20-positive, Ph-negative ALL. (Funded by the Regional Clinical Research Office, Paris, and others; ClinicalTrials.gov number, NCT00327678.)

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The outcome for adults with acute lymphoblastic leukemia (ALL) has significantly improved over the past decade, notably because of treatment with more intensive chemotherapy, similar to that used for pediatric ALL, and risk-adapted use of allogeneic hematopoietic stem-cell transplantation.[1,2] Biologic features of some ALL subtypes have also offered opportunities for targeted treatments. Although tyrosine kinase inhibitors are now used to treat Philadelphia chromosome (Ph)–positive ALL, one of the most promising new approaches relies on the use of monoclonal antibodies targeting the CD19, CD20, CD22, CD33, and CD52 surface antigens expressed by ALL blast cells.[3,4] The use of rituximab, a chimeric monoclonal antibody against CD20, has led to significant improvement in outcomes for patients with B-cell non-Hodgkin's lymphoma and, more recently, for those with Burkitt's mature B-cell lymphoma or leukemia.[5-7]

Although the majority of B cells express the CD20 antigen, it is present on only 30 to 50% of B-cell precursor ALL blasts.[3,8] We and others have observed an adverse prognostic significance of CD20 expression in adults with B-cell precursor ALL,[9,10] which prompted the incorporation of rituximab into chemotherapy regimens. Some single-group studies have suggested that adding rituximab to chemotherapy could improve the outcome for such patients.[11,12] To prospectively confirm this potential benefit, we conducted a multicenter, randomized trial evaluating the addition of rituximab to chemotherapy in patients with Ph-negative, B-lineage ALL expressing the CD20 antigen.

METHODS

STUDY DESIGN

The Group for Research on Adult Acute Lymphoblastic Leukemia 2005 (GRAALL-2005) trial was conducted between 2006 and 2014 at 56 French and 9 Swiss centers. This trial followed from the GRAALL-2003 trial, the results of which have been reported previously.[13] The design of the GRAALL-2005 trial was similar to that of the GRAALL-2003 trial, with the addition of randomized evaluation of hyperfractionated cyclophosphamide during induction and late intensification, as well as randomized evaluation of rituximab in patients with CD20-positive, B-cell precursor ALL. The rituximab addition specifically constituted the GRAALL-2005/R study, and the results are presented here. The protocol is available with the full text of this article at NEJM.org.

STUDY POPULATION

Patients in the GRAALL-2005 study were eligible for the GRAALL-2005/R study if they were 18 to 59 years of age and had newly diagnosed, Ph-negative, B-cell precursor ALL expressing CD20. Positivity for CD20 was defined as baseline expression of the CD20 antigen in more than 20% of leukemic cells. Multiparameter flow-cytometric immunophenotyping was performed locally with the use of CD45 expression as a marker to gate the ALL blast population, according to recommendations from the European LeukemiaNet.[8] Patients with Burkitt's mature B-cell lymphoma or leukemia were excluded.

Between May 2006 and September 2011, the GRAALL-2005 trial was open to both patients with CD20-negative ALL and those with CD20-positive ALL. During this period, the incidence of CD20-positive cases among all patients with Ph-negative, B-cell precursor ALL was 32%. Between September 2011 and April 2014, the trial was continued only for patients with CD20-positive ALL, in order to achieve the sample size needed for the GRAALL-2005/R study. During enrollment, 220 patients from 59 centers were randomly assigned to one of the GRAALL-2005/R study groups. Nine patients were not eligible (5 with Ph-positive ALL, 3 with CD20-negative ALL, and 1 with human immunodeficiency virus infection), and 2 patients withdrew consent. These 11 patients were excluded from the modified intention-to-treat analysis presented here, leaving 209 patients (105 in the rituximab group and 104 in the control group). Postinduction status, the numbers of patients in first remission who underwent hematopoietic stem-cell transplantation, and the numbers of patients who had a relapse or died are shown in Figure S1 in the Supplementary Appendix, available at NEJM.org.

STUDY OVERVIEW

The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines and was approved by the Institu-

tional Ethics Committee Ile-de-France VI, France. Written informed consent was obtained from all patients at trial entry. The GRAALL scientific board designed the study and made the decision to submit the manuscript for publication. Data were collected by the GRAALL investigators. Statistical analyses were performed by the second author. All the authors had full access to the data. The manuscript was written by the first two and the last two authors. No one who is not listed as an author contributed to the manuscript. All the authors vouch for the accuracy and completeness of the data and adherence to the study protocol. Rituximab (MabThera) was donated by Roche, which had no role in the study design, data collection, data analysis, or manuscript preparation.

TREATMENTS AND PROCEDURES

The GRAALL-2005 chemotherapy regimen is described in Table S1 in the Supplementary Appendix. Rituximab was given as an intravenous infusion at a dose of 375 mg per square meter of body-surface area per day during induction (days 1 and 7), salvage reinduction when needed (days 1 and 7), consolidation blocks 1, 3, 4, and 6 (4 infusions), late intensification (days 1 and 7), late consolidation (blocks 7 and 9; 2 infusions), and maintenance (6 infusions), for a total of 16 infusions (18 in the case of salvage reinduction). Each infusion of rituximab was administered after hydration and before chemotherapy. Patients received acetaminophen and dexchlorpheniramine 30 to 60 minutes before the infusion was started. When the administration of prednisone or dexamethasone was planned for the same day, the glucocorticoid was also given before the rituximab infusion. No monitoring or replacement of the serum immunoglobulin level was planned.

During the first complete remission, allogeneic hematopoietic stem-cell transplantation was offered to patients who were 55 years of age or younger if they had a suitable donor (a matched related donor or an unrelated donor with a 10/10 allele match) and were considered to be at high risk. High-risk patients were those who met one or more of the following criteria: central nervous system involvement; a white-cell count of 30×10^9 per liter or higher; a CD10-negative immature immunophenotype; *MLL* (mixed-lineage leukemia) gene rearrangement, defined as t(4;11) chromosomal translocation, *MLL-AFF1* fusion, or another *MLL* rearrangement; t(1;19) chromosomal translocation or *TCF3-PBX1* fusion; low hypodiploidy or near triploidy on karyotype or DNA index analysis; a complex karyotype, according to the criteria of Moorman and colleagues[14]; poor early peripheral-blood blast clearance, defined as a blast count higher than 1×10^9 per liter at the end of the glucocorticoid prephase; poor early bone marrow blast clearance, defined by morphologic evidence of more than 5% blasts at the end of the first week of induction chemotherapy; or late complete remission, defined by a need for salvage reinduction to achieve complete remission.[15] The minimal residual disease level was evaluated at five central laboratories on the basis of immunoglobulin or T-cell receptor gene rearrangements in bone marrow samples.[16]

STATISTICAL ANALYSIS

The primary end point of the study was event-free survival. Events were failure of complete remission induction, relapse, and death. We estimated that a sample of 220 patients, with a total of 88 events, would provide the study with 85% power to detect an increase of 20 percentage points in the rate of event-free survival (from 50% to 70%) at 2 years with the addition of rituximab to chemotherapy (hazard ratio, 0.51), on the basis of a two-sided log-rank test and a type 1 error of 5%. The total number of 88 events had been observed as of December 26, 2014. Analyses were performed according to the intention-to-treat principle, with the use of data that were updated on June 1, 2015. Overall and event-free survival rates were calculated from the date of randomization.

Secondary end points were the rate of hematologic remission, cumulative incidences of relapse and death during the first remission, overall survival, and safety. Remission rates were compared by means of Fisher's exact test. Data on time to events, except for cumulative incidences, were estimated with the Kaplan–Meier method[17] and were compared between groups by means of the log-rank test, with hazard ratios and 95% confidence intervals estimated on the basis of the Cox model.[18] Proportional-hazards assumptions were checked graphically. For estimating the cumulative incidence of relapse and the cumulative incidence of death during the first remission, deaths during the first remission and relapses, respectively, were taken into account as

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	All Patients (N=209)	Rituximab Group (N=105)	Control Group (N=104)
Age			
Median (IQR) — yr	40.2 (24.5–52.6)	39.9 (25.4–51.6)	41.5 (24.3–53.4)
≤30 yr — no. (%)	74 (35)	36 (34)	38 (37)
ECOG performance status >1 — no. (%)†	27 (13)	9 (9)	18 (17)
White-cell count ≥30×10^9/liter — no. (%)	44 (21)	21 (20)	23 (22)
CNS involvement — no. (%)‡	13 (6)	7 (7)	6 (6)
Cytogenetic features — no. (%)			
t(4;11)(q21;q23)/*MLL-AFF1*	2 (1)	1 (1)	1 (1)
t(1;19)(q23;p13)/*TCF3-PBX1*	5 (2)	3 (3)	2 (2)
Low hypodiploidy or near triploidy	20 (10)	11 (10)	9 (9)
Complex	21 (10)	10 (10)	11 (11)

* There were no significant between-group differences in the listed baseline characteristics. CNS denotes central nervous system, and IQR interquartile range.
† The Eastern Cooperative Oncology Group (ECOG) performance status is measured on a scale from 0 to 5, with higher numbers indicating increasing disability.
‡ Information about CNS involvement was missing for one patient in the rituximab group.

competing risks, with the use of the cumulative incidence curves, and were then compared between groups by means of the Gray test, whereas the Fine and Gray model was used to estimate the subdistribution hazard ratio.[19]

In post hoc analyses, we used the statistical interaction test of Gail and Simon[20] to investigate whether the treatment effect on the primary end point differed in four subgroups of interest. The treatment effect was also adjusted according to the following potential prognostic variables: age, an Eastern Cooperative Oncology Group performance status greater than 1 (on a scale from 0 to 5, with higher numbers indicating greater disability), a white-cell count of 30×10^9 per liter or higher, and central nervous system involvement. In post hoc sensitivity analyses, data on allogeneic stem-cell transplantation during the first remission were censored at the time of transplantation and then introduced as a time-dependent covariate in the multivariable model. Safety was evaluated on the basis of the incidences of grade 3 or 4 adverse events[21] and incidence rates of reported severe adverse events according to patient-years of treatment exposure. The type 1 error was fixed at the 5% level. All tests were two-sided. All analyses were performed with SAS software, version 9.3 (SAS), or R software, version 2.14.0 (survival and cmprsk packages).

RESULTS

CHARACTERISTICS OF THE PATIENTS

Pretreatment characteristics were well balanced between the two groups (Table 1). The median percentage of CD20-positive blasts was 66% (range, 20 to 100), and the percentages were evenly distributed between the groups, as shown in Figure S2 in the Supplementary Appendix.

OUTCOMES

As of June 1, 2015, after a median follow-up of 30 months, a total of 101 patients (48%) had had at least one event: 44 patients (42%) in the rituximab group and 57 (55%) in the control group. There were 17 induction failures (8 in the rituximab group and 9 in the control group), 57 relapses (22 and 35, respectively), and 27 deaths during remission (14 and 13, respectively). Two patients, both in the rituximab group, were lost to follow-up early (i.e., during the first 12 months of follow-up).

With respect to the primary study end point, patients assigned to the rituximab group had longer event-free survival than those assigned to the control group (hazard ratio, 0.66; 95% confidence interval [CI], 0.45 to 0.98; P=0.04). Figure 1A shows the event-free survival curves in both groups over time.

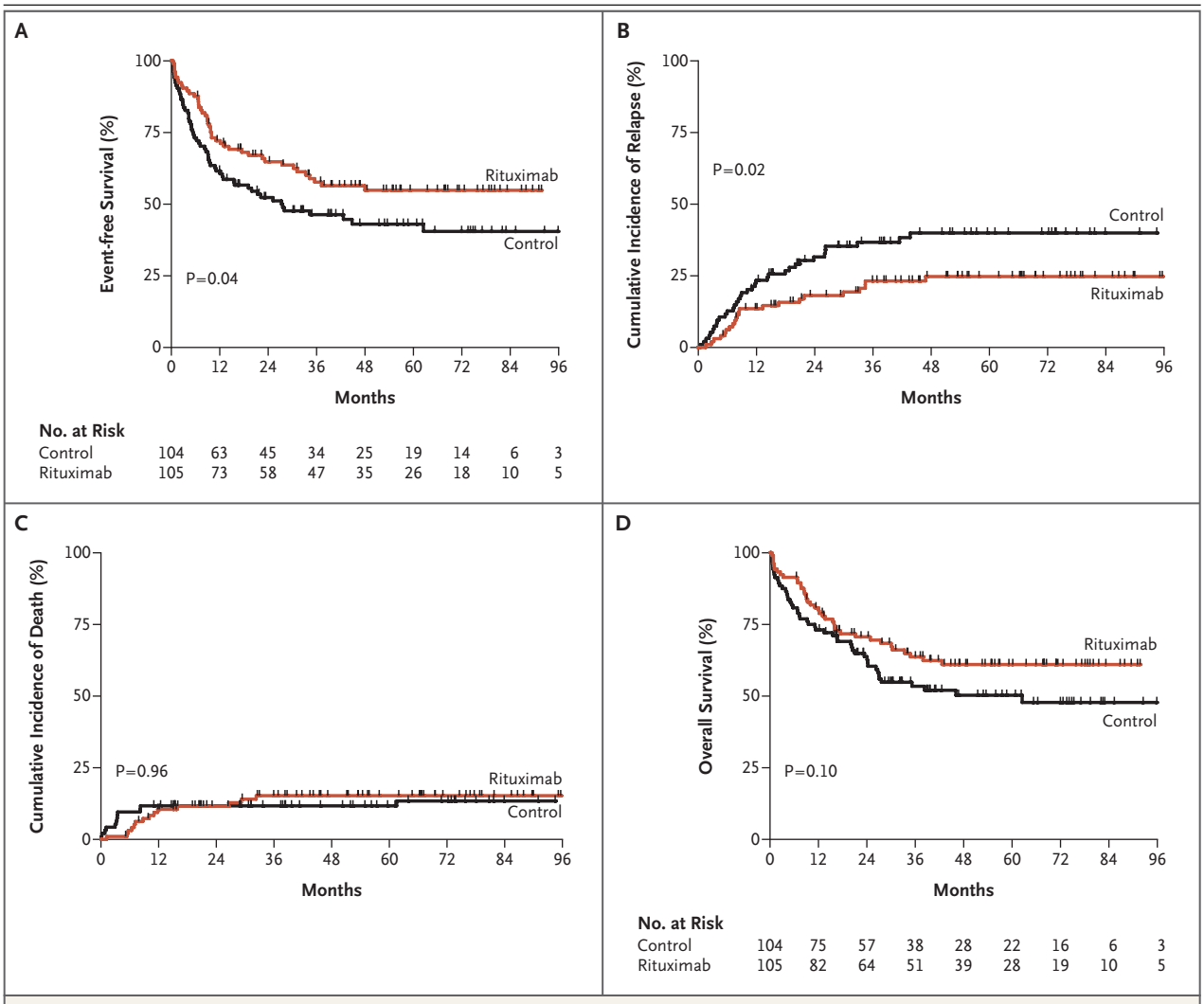

Figure 1. Clinical Outcomes in the Rituximab and Control Groups.

Panel A shows the rate of event-free survival over time, which was estimated at 65% (95% confidence interval [CI], 56 to 75) in the rituximab group versus 52% (95% CI, 43 to 63) in the control group at 2 years and at 55% (95% CI, 46 to 66) and 43% (95% CI, 34 to 55), respectively, at 4 years. Panel B shows the cumulative incidence of relapse over time; at 2 years and 4 years, respectively, the cumulative incidence was estimated at 18% (95% CI, 11 to 27) and 25% (95% CI, 16 to 35) in the rituximab group versus 32% (95% CI, 22 to 42) and 41% (95% CI, 30 to 51) in the control group. Panel C shows the cumulative incidence of death during the first remission; at 2 years, the cumulative incidence was estimated at 12% (95% CI, 6 to 19) in the rituximab group and 12% (95% CI, 6 to 19) in the control group; at 4 years, these estimates were 16% (95 CI, 9 to 24) and 12% (95% CI, 6 to 19), respectively. Panel D shows the rate of overall survival, which was estimated at 71% (95% CI, 62 to 80) in the rituximab group and 64% (95% CI, 55 to 74) in the control group at 2 years and at 61% (95% CI, 52 to 72) and 50% (95% CI, 41 to 62), respectively, at 4 years. Censoring of data is indicated by the vertical bars.

The difference in event-free survival was mostly due to a lower incidence of relapse in the rituximab group, with a subdistribution hazard ratio of 0.52 (95% CI, 0.31 to 0.89; P=0.02) (Fig. 1B). In contrast, the cumulative incidence of death during the first remission was similar in the two groups, with a subdistribution hazard ratio of 0.98 (95% CI, 0.45 to 2.12; P=0.96) (Fig. 1C). This benefit in event-free survival did not translate into significantly longer overall survival (hazard ratio, 0.70; 95% CI, 0.46 to 1.07; P=0.10) (Fig. 1D).

Early peripheral-blood and bone marrow blast clearance did not differ significantly between the two groups. After induction with or without salvage reinduction, the rate of complete remis-

Table 2. Response to Initial Therapy.*

Variable	All Patients (N=209)	Rituximab Group (N=105)	Control Group (N=104)	P Value
Early response to therapy — no. (%)				
Poor peripheral-blood blast clearance	34 (16)	20 (19)	14 (13)	0.35
Poor bone marrow blast clearance	87 (42)	46 (44)	41 (39)	0.58
Response to induction — no. (%)				
Complete remission				
Without salvage reinduction	186 (89)	95 (90)	91 (88)	0.52
With or without salvage reinduction	191 (91)	97 (92)	94 (90)	0.63
Resistant disease	2 (2)	1 (1)	1 (1)	
Death during induction	16 (8)	7 (7)	9 (9)	
MRD $<10^{-4}$ bone marrow blasts — no./total no. (%)				
After first induction course	54/85 (64)	32/49 (65)	22/36 (61)	0.82
After first consolidation phase	70/80 (88)	42/46 (91)	28/34 (82)	0.31
High-risk ALL — no. (%)†	140 (67)	73 (70)	67 (64)	
Allogeneic SCT during first complete remission — no. (%)	57 (27)	36 (34)	21 (20)	

* MRD denotes minimal residual disease, and SCT stem-cell transplantation.
† High-risk acute lymphoblastic leukemia (ALL) was determined according to protocol-specified criteria.

sion was 92% in the rituximab group (97 of 105 patients) and 90% in the control group (94 of 104 patients). Minimal residual disease levels were evaluated in 85 patients (41%) and 80 patients (38%) after the first induction course and the first consolidation phase, respectively. The percentages of patients with minimal residual disease levels lower than 10^{-4} (i.e., <1 bone marrow blast in 10,000 normal cells) were 65% in the rituximab group and 61% in the control group after the first induction course and 91% and 82%, respectively, after the first consolidation phase (Table 2).

Overall, the proportion of high-risk patients who were eligible for allogeneic transplantation during the first complete remission, as specified by the protocol, was similar in the two groups. Nevertheless, a higher proportion of patients in the rituximab group underwent transplantation during the first remission (34%, vs. 20% in the control group) (Table 2). Among the 21 patients in the control group who received a transplant, 6 had a relapse and 5 died; 2 of the deaths occurred during remission. Among the 36 patients in the rituximab group who received a transplant, 5 had a relapse and 14 died; 9 of the deaths occurred during remission. The causes of post-transplantation deaths during remission are provided in Table S2 in the Supplementary Appendix. In a post hoc sensitivity analysis with censoring of data at the time of transplantation for patients who received an allogeneic transplant during the first remission, event-free survival was still longer in the rituximab group than in the control group (hazard ratio, 0.59; 95% CI, 0.37 to 0.93; P=0.02), as was overall survival (hazard ratio, 0.55; 95% CI, 0.34 to 0.91; P=0.02). The incidence of relapse remained lower in the rituximab group (subdistribution hazard ratio, 0.49; 95% CI, 0.27 to 0.89; P=0.02), and the cumulative incidence of death during the first remission was similar to that in the control group (subdistribution hazard ratio, 0.79; 95% CI, 0.43 to 0.1.47; P=0.46).

PROGNOSTIC FACTORS AND SUBGROUP ANALYSES

In addition to randomized assignment to the control group, factors associated with significantly shorter event-free survival were older age, central nervous system involvement, and a higher white-cell count at diagnosis. Together with the assignment to the control group, all these factors remained significantly associated with shorter event-free survival in a multivariate analysis (Table S3 in the Supplementary Appendix). Post hoc subgroup analyses of the treatment effect

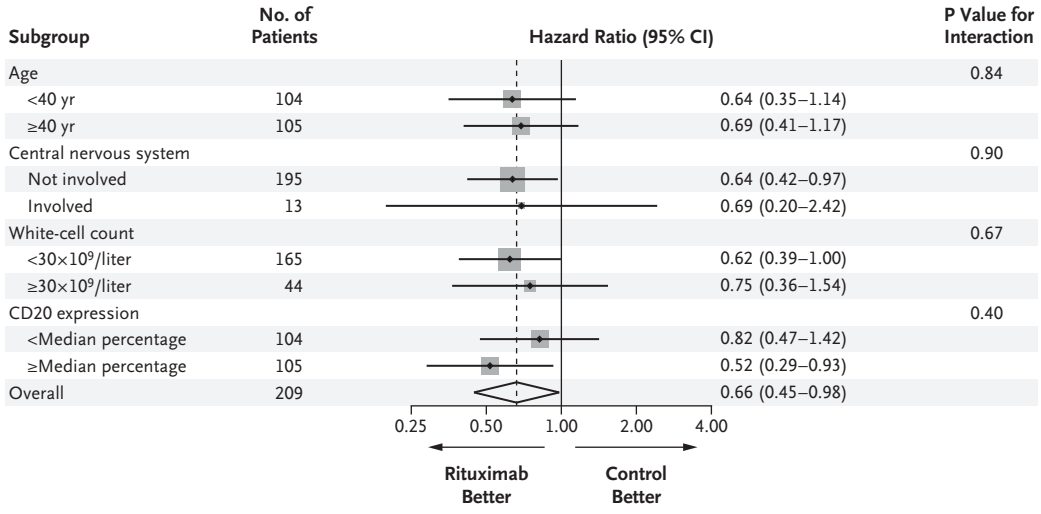

Figure 2. Effect of Rituximab Treatment in Subgroups of Patients.

Shown are the hazard ratios for failure of complete remission induction, relapse, or death among patients receiving rituximab added to chemotherapy as compared with those receiving chemotherapy alone in subgroups defined according to age, presence or absence of central nervous system involvement, white-cell count, and level of CD20 antigen expression at baseline. No evidence of a significant interaction was found. The gray squares indicate hazard ratios, with the size of each square proportional to the number of patients in the subgroup. Information about central nervous system involvement was missing for one patient in the rituximab group. The diamond and the dashed line indicate the overall hazard ratio for the whole patient cohort.

showed no treatment-by-subgroup interaction, with hazard ratios consistently favoring the rituximab group across subgroups defined by age, presence or absence of central nervous system involvement, white-cell count, and CD20 expression level (Fig. 2). A more pronounced effect of rituximab was observed in patients with higher levels of CD20 expression, although the difference was not significant.

ADHERENCE TO RITUXIMAB TREATMENT AND SAFETY

The percentages of patients receiving all planned rituximab infusions during the successive treatment phases were 94% (99 of 105 patients) for induction, 84% (77 of 92) for consolidation block 1, 77% (71 of 92) for consolidation block 3, 88% (70 of 80) for consolidation block 4, 80% (56 of 70) for consolidation block 6, 85% (45 of 53) for late intensification, 76% (35 of 46) for consolidation block 7, and 79% (34 of 43) for consolidation block 9 (Fig. S3 in the Supplementary Appendix).

Overall, 246 severe adverse events were reported in 124 patients (67 patients with 1 event, 26 with 2 events, 13 with 3 events, and 18 with 4 or more events). The overall incidence of severe events did not differ significantly between the two groups (Table 3). Although infectious events were slightly more frequent in the rituximab group, the difference was not significant. Among the 16 patients who had severe allergic events (of which all but 1 was related to asparaginase administration), only 2 were in the rituximab group ($P=0.002$). When all asparaginase-containing treatment phases were analyzed separately, this better side-effect profile did not significantly affect adherence to the planned asparaginase therapy until at least the end of the late intensification phase. However, more patients in the control group than in the rituximab group required a switch to the erwinia form of asparaginase during the course of therapy, as specified by the protocol in cases of clinical allergic reaction to native *Escherichia coli* asparaginase (Fig. S4 in the Supplementary Appendix). All reported grade 3 or 4 adverse events are listed in Table S4 in the Supplementary Appendix.

DISCUSSION

This randomized study showed that the addition of rituximab to standard chemotherapy signifi-

cantly improved event-free survival among adults with CD20-positive ALL. This gain was explained by a reduction in the cumulative incidence of relapse, with no significant increase in toxic effects or the cumulative incidence of death during the first remission. Although more patients in the rituximab group than in the control group underwent allogeneic stem-cell transplantation during the first remission, the reduced incidence of relapse and improvement in event-free survival cannot be clearly explained by a potential benefit of transplantation for three reasons. First, most of the patients who died from the transplantation procedure were in the rituximab group. Second, the reduction in the relapse rate and the increase in event-free survival and overall survival remained significant in sensitivity analyses with censoring data for patients who underwent transplantation. Finally, the treatment effect with rituximab was also observed in an analysis adjusted for transplantation as a time-dependent covariate.

A direct effect of rituximab, mediated by its binding to leukemic cells, is suggested by the more pronounced benefit observed in patients with higher levels of CD20 expression on their leukemic blasts (Fig. 2). However, an indirect mechanism might be indicated by the unexpected observation that fewer patients in the rituximab group than in the control group had allergic reactions to asparaginase, suggesting that patients treated with rituximab may have received a higher cumulative dose of asparaginase during their treatment course. Such a protective effect of rituximab could be related, at least theoretically, to inhibition of the production of anti-asparaginase antibodies, which are known to be involved in these allergic events,[22,23] through the removal of normal B cells. However, this hypothesis is not supported by the similar adherence to planned asparaginase treatment in the two study groups during most of the asparaginase-containing treatment phases (Fig. S4 in the Supplementary Appendix). One could nevertheless argue that the rate of development of clinically silent anti-asparaginase antibodies, which may also impair the efficacy of asparaginase therapy, may have been lower in the rituximab group.

In the present study, rituximab was given during all treatment phases, including maintenance, for a total of 16 to 18 infusions. This schedule of administration was mostly empirical, based on previous experience with rituximab treatment in patients with non-Hodgkin's lymphoma. For instance, in a single-group study at the M.D. Anderson Cancer Center, rituximab was given at the same dose but for a total of only 12 doses.[11] Further studies would help define an adequate schedule of administration. Nevertheless, in the present study, repeated rituximab administration reduced the incidence of relapse without significantly affecting the rate of complete remission or the quality of complete remission in terms of minimal residual disease levels. Prolonged administration may thus play a role in the beneficial outcome that we observed.

Another unanswered question is whether the addition of rituximab to chemotherapy might also benefit patients with a level of CD20 expression that is lower than the 20% cutoff used to date. Using this cutoff, we observed that 32% of cases of Ph-negative, B-cell precursor ALL would be defined as CD20-positive, which is similar to the proportion we previously reported, in the GRAALL-2003 trial.[9] In the M.D. Anderson Cancer Center study, which used the same 20% cutoff, the proportion of CD20-positive cases was 47%.[10] However, approximately 20% of the patients in that study had Ph-positive ALL, and in that subgroup, the proportion of CD20-positive cases appeared to be slightly higher. Several

Table 3. Severe Adverse Events.*

Event	All Patients (N = 209)	Rituximab Group (N = 105)	Control Group (N = 104)	P Value†
	no. of events (incidence rate)			
No. of patient-yr	261	133	128	
Infection	126	71	55	
Laboratory abnormalities	45	22	23	
Allergy	16	2 (2)	14 (11)	0.002
Neurologic event	12	6	6	
Pulmonary event	8	5	3	
Coagulopathy	6	3	3	
Cardiac event	5	1	4	
Gastrointestinal event	5	3	2	
Other	23	15	8	
Total	246	128 (96)	118 (92)	0.72

* The incidence rate is the number of events per 100 patient-years of exposure to treatment. Incidence rates are shown for the total number of events and for allergy, which was the only adverse event for which there was a significant difference in rates between the rituximab group and the control group.
† P values are for the comparison of incidence rates (two-sided test).

studies have suggested that rituximab might also benefit patients with CD20 expression at a level below 20%. Thomas et al. reported that the use of a 10% cutoff in their study had significant prognostic value.[10] Another finding was the upregulation of CD20 expression during induction chemotherapy in ALL blasts that were classified as CD20-negative at baseline.[24] On the basis of these findings, the U.K. NCRI Adult ALL group is currently conducting a randomized trial (UKALL14; ClinicalTrials.gov number, NCT01085617) to assess the addition of rituximab to chemotherapy irrespective of the CD20 expression level. In patients with low or very low CD20 expression levels at baseline, the use of higher-affinity anti-CD20 antibodies, such as obinutuzumab or ofatumumab, might offer an attractive alternative.

Our study has several limitations related to the multiple comparisons required for prespecified secondary end points and post hoc analyses. Despite these limitations, the results provide evidence of a beneficial effect of the addition of rituximab to chemotherapy in adults with CD20-positive, Ph-negative, B-cell precursor ALL. Other surface antigens, such as CD19 or CD22, which ALL blasts express more frequently than they express CD20, may also be targeted by various antibodies or chimeric antigen receptor T cells that have already shown efficacy in patients with relapsed or refractory ALL.[25-29]

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

APPENDIX

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REFERENCES

1. Gökbuget N, Hoelzer D. Treatment of adult acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program 2006;2006:133-41.
2. Dombret H, Cluzeau T, Huguet F, Boissel N. Pediatric-like therapy for adults with ALL. Curr Hematol Malig Rep 2014; 9:158-64.
3. Gökbuget N, Hoelzer D. Treatment with monoclonal antibodies in acute lymphoblastic leukemia: current knowledge and future prospects. Ann Hematol 2004; 83:201-5.
4. DeAngelo DJ. The use of novel monoclonal antibodies in the treatment of acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program 2015;2015: 400-5.
5. Oriol A, Ribera JM, Bergua J, et al. High-dose chemotherapy and immunotherapy in adult Burkitt lymphoma: comparison of results in human immunodeficiency virus-infected and noninfected patients. Cancer 2008;113:117-25.
6. Hoelzer D, Walewski J, Döhner H, et al. Improved outcome of adult Burkitt lymphoma/leukemia with rituximab and chemotherapy: report of a large prospective multicenter trial. Blood 2014;124: 3870-9.
7. Ribrag V, Koscielny S, Bosq J, et al. Rituximab and dose-dense chemotherapy for adults with Burkitt's lymphoma: a randomised, controlled, open-label, phase 3 trial. Lancet 2016;387:2402-11.
8. Béné MC, Nebe T, Bettelheim P, et al. Immunophenotyping of acute leukemia and lymphoproliferative disorders: a consensus proposal of the European LeukemiaNet Work Package 10. Leukemia 2011; 25:567-74.
9. Maury S, Huguet F, Leguay T, et al. Adverse prognostic significance of CD20 expression in adults with Philadelphia chromosome-negative B-cell precursor acute lymphoblastic leukemia. Haematologica 2010;95:324-8.
10. Thomas DA, O'Brien S, Jorgensen JL, et al. Prognostic significance of CD20 expression in adults with de novo precursor B-lineage acute lymphoblastic leukemia. Blood 2009;113:6330-7.
11. Thomas DA, O'Brien S, Faderl S, et al. Chemoimmunotherapy with a modified hyper-CVAD and rituximab regimen improves outcome in de novo Philadelphia chromosome-negative precursor B-lineage acute lymphoblastic leukemia. J Clin Oncol 2010;28:3880-9.
12. Hoelzer D, Gökbuget N. Chemoimmunotherapy in acute lymphoblastic leukemia. Blood Rev 2012;26:25-32.
13. Huguet F, Leguay T, Raffoux E, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. J Clin Oncol 2009;27:911-8.
14. Moorman AV, Harrison CJ, Buck GA, et al. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII/Eastern Cooperative Oncology Group (ECOG) 2993 trial. Blood 2007;109:3189-97.

15. Dhédin N, Huynh A, Maury S, et al. Role of allogeneic stem cell transplantation in adult patients with Ph-negative acute lymphoblastic leukemia. Blood 2015; 125:2486-96.
16. Beldjord K, Chevret S, Asnafi V, et al. Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. Blood 2014;123:3739-49.
17. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.
18. Cox C. Multinomial regression models based on continuation ratios. Stat Med 1988;7:435-41.
19. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94: 496-509.
20. Gail M, Simon R. Testing for qualitative interactions between treatment effects and patient subsets. Biometrics 1985;41: 361-72.
21. Trotti A, Colevas AD, Setser A, et al. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. Semin Radiat Oncol 2003;13:176-81.
22. Woo MH, Hak LJ, Storm MC, et al. Hypersensitivity or development of antibodies to asparaginase does not impact treatment outcome of childhood acute lymphoblastic leukemia. J Clin Oncol 2000;18:1525-32.
23. Avramis VI, Sencer S, Periclou AP, et al. A randomized comparison of native Escherichia coli asparaginase and polyethylene glycol conjugated asparaginase for treatment of children with newly diagnosed standard-risk acute lymphoblastic leukemia: a Children's Cancer Group study. Blood 2002;99:1986-94.
24. Dworzak MN, Schumich A, Printz D, et al. CD20 up-regulation in pediatric B-cell precursor acute lymphoblastic leukemia during induction treatment: setting the stage for anti-CD20 directed immunotherapy. Blood 2008;112:3982-8.
25. Chevallier P, Huguet F, Raffoux E, et al. Vincristine, dexamethasone and epratuzumab for older relapsed/refractory CD22+ B-acute lymphoblastic leukemia patients: a phase II study. Haematologica 2015; 100(4):e128-31.
26. Kantarjian H, Thomas D, Jorgensen J, et al. Results of inotuzumab ozogamicin, a CD22 monoclonal antibody, in refractory and relapsed acute lymphocytic leukemia. Cancer 2013;119:2728-36.
27. Topp MS, Gökbuget N, Stein AS, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. Lancet Oncol 2015;16:57-66.
28. Fry TJ, Mackall CL. T-cell adoptive immunotherapy for acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program 2013;2013:348-53.
29. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med 2014; 371:1507-17.