Randomized Phase II Study of Interleukin-12 in Combination with Rituximab in Previously Treated Non-Hodgkin’s Lymphoma Patients

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Abstract

Purpose: Rituximab is a chimeric antibody that induces B-cell apoptosis and recruits immune effector cells to mediate cell lysis. Interleukin-12 (IL-12) facilitates cytolytic responses by T cells and natural killer cells. This phase II study was done to determine the efficacy and toxicity of IL-12 in combination with rituximab in patients with B-cell non-Hodgkin’s lymphoma (NHL).

Experimental Design: Fifty-eight patients with histologically confirmed relapsed B-cell NHL were randomized to receive concurrent treatment with rituximab and IL-12 (arm A) or rituximab with subsequent treatment with IL-12 after documented nonresponse or progression after rituximab (arm B). Treatment consisted of 375 mg/m2 rituximab on days 1, 8, 15, and 22 and 300 ng/kg IL-12 given s.c. twice weekly starting on day 2 for arm A or upon progression for arm B.

Results: The overall response rate was 37% (11 of 30) in arm A and 52% (13 of 25) in arm B. All of the responses seen in arm B occurred while patients received rituximab, and no responses occurred during treatment with subsequent IL-12. The median duration of response was 16 months for arm A and 12 months for arm B. Biopsy specimens were serially obtained in a subset of patients and showed that changes in gene expression were different when cells from the peripheral blood were compared with cells from lymph node biopsies.

Conclusions: The concomitant use of IL-12 and rituximab had modest disease activity in patients with B-cell NHL, but the sequential administration of IL-12 after rituximab did not result in additional clinical responses.

Materials and Methods

Patient eligibility: To be eligible for participation in this study, patients were older than 18 years of age with histologic proof of CD20-positive indolent B-cell NHL. Due to data obtained in the phase I trial suggesting efficacy in mantle cell lymphoma (MCL), patients with this histology were also eligible, but the study was stratified for this histology.
The histology of the most recent biopsy specimen was centrally reviewed and patients were treated at centers affiliated with the North Central Cancer Treatment Group. All patients were required to have a life expectancy ≥12 weeks, adequate laboratory values, and an Eastern Cooperative Oncology Group performance status of 0 to 1. Patients were eligible if they had previously received rituximab; however, patients had to have received rituximab ≥12 months previously. Patients previously treated with IL-12, HIV-positive patients, patients with autoimmune diseases, and patients requiring concurrent steroid therapy were not eligible. All patients were required to give informed consent, and the Institutional Review Board of the respective North Central Cancer Treatment Group sites approved the study.

**Study design.** Patients were randomized to one of two treatment arms: concurrent treatment with rituximab + IL-12 (arm A) or rituximab with subsequent treatment with IL-12 after documented nonresponse, defined as less than a partial response (arm B). An accrual goal of 45 patients in each arm was planned to independently determine the primary end point of confirmed response (complete or partial). Secondary end points of the study were the time to progression, duration of response, and overall survival. Treatment consisted of 375 mg/m² rituximab given on days 1, 8, 15, and 22 and 300 ng/kg IL-12 given s.c. weekly starting on day 2 for arm A or upon progression for arm B. IL-12 was given s.c. twice weekly for a maximum of 24 weeks. IL-12 was obtained from the Clinical Trial Evaluation Program of the National Cancer Institute, and rituximab was obtained commercially.

**Toxicity and response evaluation.** No modifications in the dose of rituximab were permitted. Rituximab was discontinued if patients developed grade 3 or 4 toxicity. To prevent infusion-related reactions, all patients received acetaminophen and diphenhydramine at the time rituximab was given. Steroids were not given with rituximab. The dose of IL-12 was adjusted if patients experienced adverse effects. For severe constitutional symptoms, grade ≥2 hepatic dysfunction, gastrointestinal bleeding, or a serum creatinine ≥2 times the baseline value, IL-12 was held for up to 21 days until resolved and then decreased to 100 ng/kg.

In arm A, patients who had stable disease or were responding continued to receive IL-12 twice weekly at the physician’s discretion until progression or up to 24 weeks. In arm B, patients responding to treatment at the time of the evaluation at 12 weeks were followed without treatment until disease progression and were then treated with IL-12. Patients in arm B with stable disease or progressing at 12 weeks were treated with IL-12. Patients subsequently having objective progression of disease or clinical deterioration were taken off study. Patient responses to therapy were assessed using the International Working Group recommendations for response criteria for NHL (8).

**Gene expression arrays.** Total RNA was isolated from lymphoma biopsies by homogenizing the tumor in TRIzol reagent (Life Technologies, Gaithersburg, MD). RNA was extracted and purified using commercial affinity resin column kits (Qiagen, Chatsworth, CA). cDNA was prepared from a total of 10 µg of RNA. The purified cDNA was used as a template for in vitro transcription reaction for the synthesis of biotinylated cRNA using RNA transcript labeling reagent (Affymetrix, Santa Clara, CA). Labeled cRNA was then fragmented and hybridized onto the U133Plus2 array. After hybridization, the arrays were washed and then stained with streptavidin-phycocerythrin (Molecular Probes, Eugene, OR). The arrays were then scanned using the GeneChip Scanner 3000 (Affymetrix). GeneSpring (Agilent Technologies, Palo Alto, CA) and Microsoft Excel were used for data analysis. Expression values were compared between individual samples. The clustering of gene expression values was done using the standard correlation as the similarity measure.

**Intratumoral T-cell repertoire after therapy with IL-12 and rituximab.** RNA extraction was done using a Rapid Total RNA Isolation kit, and the Mayo Molecular Biology Core Facility synthesized the necessary primers. All primers were purified and labeled with 6-carboxyfluorescein at the 5’ end. The panel of Vα-specific primers included 25 primers specific for the individual Vα genes, and the panel of Vβ-specific primers included 34 primers specific for the individual Vβ subfamilies. Nested PCR primer extension using the 6-carboxyfluorescein–labeled Cα and Cβ primers was then done. Spectratype analysis was done by mixing the 6-carboxyfluorescein–labeled product, formamide gel loading buffer, and Genescan-500 TAMRA internal lane size standards. This was run on a 4.75% polyacrylamide urea denaturing gel in an Applied Biosystems model 377 WTR DNA-sequencing machine. Product length determination was done using the Prism Genescan software package (Applied Biosystems, Foster City, CA), and data analysis was done using the Genotype 2.1 software package (Applied Biosystems, Foster City, CA). The diversity of the T-cell receptor repertoire was assessed based on the total number of peaks representing individual CDR3 lengths, the proportion of those peaks observed in the repertoire that represent productively rearranged V gene region primers, and the distribution of CDR3 lengths to a normal distribution using the Shapiro-Wilk test.

Other analyses, such as antibody-dependent cellular cytotoxicity, cytokine production, or tumor immune cell infiltration, were not done in this study as these assays were done in the phase I clinical trial of rituximab and IL-12.

**Statistical methods.** This was a randomized single-stage phase II trial with interim analyses. The same study design was used for each treatment arm and evaluated independently, with different definitions of success for each arm. For each treatment arm, a modified Simon optimal design was used requiring a minimum of 21 and maximum of 45 evaluable patients to independently test the null hypothesis that the success rate is at most 50% in each treatment arm (reflecting the anticipated response rate to rituximab alone). Success was defined as an objective response by 12 weeks in arm A and by 24 weeks in arm B due to the sequential nature of that arm’s treatment regimen. This study design had 90% power to detect an effective treatment if the true success rate was at least 70% versus the null hypothesis that it was at most 50%, with 10% type I error rate. For each treatment arm, 21 evaluable patients were accrued. If at most 11 of these first 21 patients were successes, then we considered this evidence that the treatment regimen was insufficiently active. If ≥12 of these 21 patients were successes, then an additional 24 patients would be accrued for a total of 45 evaluable patients per arm. The overall decision criteria in each arm required at least 27 successes to be observed of the 45 evaluable patients for that treatment regimen to be considered promising in this patient population.

The number of successes was assumed to be binomially distributed, where the success rates were calculated along with 95% confidence intervals. Standard paired comparisons methodologies (paired t tests, Wilcoxon signed rank tests, and Fisher’s exact tests for interval, ordinal, and nominal level data, respectively) were used to compare the average intra-patient variables between baseline values and posttreatment. The time to progression, duration of response, and overall survival was estimated using the Kaplan-Meier method, and univariate associations were determined using the log-rank test.

**Results**

**Patient characteristics.** Fifty-eight patients with histologically confirmed CD20+ low-grade B-cell NHL, felt to be incurable with standard therapy, were enrolled in this phase II study between October 30, 2001 and April 19, 2004. All patients had measurable disease at study entry. The patient characteristics for all registered patients are presented in Table 1. One patient went off study before receiving treatment and was classified as a cancel. On pathology review, one patient on arm A and one patient on arm B had evidence for transformed lymphoma and were deemed ineligible for the study. The two ineligible patients were treated and used in the toxicity analyses and on-study tables. However, they were not used for end point analyses (response, time to progression, or survival).
The median age of the patients was 63 years (range, 37-85 years), and the median number of previous therapies was 1 (range, 1-7). All eligible patients had been previously treated, and 25 of the 55 patients (45%) accrued to the study had received prior rituximab therapy. Prior rituximab use was not a stratification factor, resulting in a modest imbalance between the two arms with 53% of arm A patients having received rituximab before this trial versus 36% of arm B patients (P = 0.2). This same level of difference in prior rituximab use was also observed when only evaluating the first 21 evaluable patients in each arm, which were evaluated for the purposes of the interim analyses.

**Toxicity.** The combination of IL-12 and rituximab was reasonably well tolerated (see Fig. 1). As expected, there were more toxicities seen when IL-12 was given concomitantly with rituximab compared with the agents given sequentially. On arm A, 13 (42%) patients had a maximum of grade 3, and 5 (16%) patients had grade 4 toxicities versus 5 (20%) patients on arm B with grade 3 toxicities (see Fig. 2). Of the five patients with grade 4 toxicity, one had grade 4 anemia; one patient had grade 4 neutropenia and leukopenia; one had neutropenia; one had grade 4 fatigue; and one had grade 4 leukopenia, infection, and bone marrow depression.

**Clinical responses.** Of all 30 evaluable patients accrued to arm A across all histologies (Table 2), 11 responses (37%) were observed. In arm B, 13 responses were seen in all 25 evaluable patients (52%); however, the responses occurred while patients were on rituximab alone, and there were no responses associated with subsequent treatment with IL-12. These responders in each of the arms were similar to each other in terms of stage of disease at study entry, best response achieved to previous therapy, risk group defined by the international prognostic factor index, and performance status. The only notable differences in baseline characteristics were that 6 of 11 responders on arm A had prior rituximab therapy versus 3 of 13 responders on arm B. This difference was not statistically significant, but sample sizes were also quite limited. In addition, 8 of 11 responders on arm A were female versus 5 of 13 on arm B.

Overall, the decision criteria for the interim analysis for each arm of this study was not met, where only 8 of the first 21 patients on arm A and 10 of the first 21 patients on arm B had a response to treatment. When we focused on the first 21 non-MCL patients in each treatment arm, six responses were seen on arm A and 11 on arm B (which were all attributed to rituximab therapy). Therefore, each treatment arm was subsequently closed to accrual. The only exception was the decision to continue accrual of only MCL patients and only to arm A. The original design allowed for additional accrual of MCL patients until 20 were accrued to each arm. When both treatment arms were closed due to early evidence of lack of sufficient activity across all histologies, the MCL patients were still allowed to accrue. This was based on early promising response data for these patients as well as data from the phase I trial. Thus, a higher number of MCL patients were accrued to arm A (7 versus 2 in arm B). Of these 7 MCL patients accrued to arm A, 3 responses (43%) were observed.

The median follow-up on all living patients was 26 months (range, 14.5-41.9 months), and all of the patients have since completed treatment. At the time of these analyses, only 8 of the first 21 patients on arm A and 10 of the first 21 patients on arm B had a response to treatment. When we focused on the first 21 non-MCL patients in each treatment arm, six responses were seen on arm A and 11 on arm B (which were all attributed to rituximab therapy). Therefore, each treatment arm was subsequently closed to accrual. The only exception was the decision to continue accrual of only MCL patients and only to arm A. The original design allowed for additional accrual of MCL patients until 20 were accrued to each arm. When both treatment arms were closed due to early evidence of lack of sufficient activity across all histologies, the MCL patients were still allowed to accrue. This was based on early promising response data for these patients as well as data from the phase I trial. Thus, a higher number of MCL patients were accrued to arm A (7 versus 2 in arm B). Of these 7 MCL patients accrued to arm A, 3 responses (43%) were observed.

<table>
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<th>Table 1. Patient characteristics</th>
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<tr>
<td>Arm A (n = 32), n (%)</td>
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<td><strong>Age, median (range)</strong></td>
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Although the overall response rate with rituximab plus IL-12 did not seem different to what would have been expected with rituximab alone, the time to progression results seemed similar or even improved when compared with that seen in the pivotal study of rituximab alone (9). As shown in Fig. 3, the median time to progression in arm A was 12 months [95% confidence interval (95% CI), 4-25]. The median time to progression on arm B was also 12 months (95% CI, 6-27) for initial treatment with rituximab, and the median time to discontinuation of all treatment (or treatment failure) for rituximab plus subsequent IL-12 was 19 months (95% CI, 7-25). McLaughlin et al. (9) in the pivotal study of four doses of rituximab also focused on time to progression in responders (median, 12.5 months; 95% CI, 11-16). In the present study, the median time to initial progression for responders was 19.1 months for arm A (95% CI, 11.2, not reached) and 15.1 months for arm B (95% CI, 9.2, not reached). Again, responses in arm B were attributed to rituximab alone (no formal comparison between treatment arms was conducted given the fact that the number of patients in each group was very limited and such a comparison is inappropriate in this type of trial). The median duration of response was 16 months (95% CI, 6, not reached) in arm A and 12 months (95% CI, 6, not reached) in arm B, when calculated from date of documented response. The median
overall survival for both treatment arms has not yet been reached (Fig. 3).

Quality of life. Quality of life was measured on 45 patients (arm A = 25 and arm B = 20) using the Functional Assessment of Cancer Therapy/Biologic Response Modifier questionnaire. Mean patient quality of life was similar for the two arms at baseline, including the Functional Assessment of Cancer Therapy/Biologic Response Modifier total score (arm A = 78.7, arm B = 79.5), the trial outcome index (arm A = 76.6, arm B = 80.0) and physical well-being (arm A = 81.8, arm B = 88.4), social/family well-being (arm A = 86.1, arm B = 79.8), emotional (arm A = 79.8, arm B = 77.1), and functional well-being (arm A = 71.6, arm B = 76.6) subscales. Two subscales at the 6-month assessment had differences that favored arm A: Functional Assessment of Cancer Therapy/Biologic Response Modifier total score (arm A = 84.3, arm B = 76.7), trial outcome index (arm A = 81.1, arm B = 76.2), physical well-being (arm A = 85.1, arm B = 84.4), social/family well-being (arm A = 94.0, arm B = 81.9; P = 0.04), emotional well-being (arm A = 87.6, arm B = 72.9; P = 0.05), and functional well-being (arm A = 82.5, arm B = 71.7) subscales. These findings showed that the addition of IL-12 to rituximab did not have a significantly negative effect on the quality of life of patients treated in this study; instead, there was a trend toward improved quality of life in patients treated up front with both rituximab and IL-12 versus rituximab alone. Interestingly, the 6-month evaluations showed a continued similarity in the physical well-being measures, but a borderline significant improvement in the social and emotional well-being subscales.

Changes in gene expression after treatment with IL-12 and rituximab. To evaluate changes in gene expression in response to IL-12 and rituximab, we did a tumor biopsy before and after initiating therapy. As this was a cooperative group study conducted through the North Central Cancer Treatment Group, biopsies were only done on patients treated at Mayo Clinic, Rochester, MN. Of the 52 patients treated on the phase II study, eight patients had matched tumor biopsies and peripheral blood specimens obtained before therapy and again 2 weeks after treatment was started. Six of the patients received IL-12 plus rituximab, whereas two received rituximab alone. Gene expression array analysis was done on RNA isolated from cells from involved lymph nodes and from peripheral blood mononuclear cells.

Specimens from the peripheral blood of patients who received IL-12 in combination with rituximab showed a 3-fold increase in the expression of multiple genes known to be up-regulated by IL-12 signaling, including IFN-γ, CXCL10 (inducible protein-10), IFIT2 and IFIT4 (IFN-induced protein with tetratricopeptide repeats 2 and 4), IL-8, and CXCL2 (macrophage inflammatory protein-2). These increases in gene expression were not seen in the peripheral blood of patients who received rituximab alone. Furthermore, the significant changes seen in cells obtained from the peripheral blood were not seen in cells obtained from lymph nodes involved by lymphoma, despite the samples being obtained from the same patient on the same day.

To address whether IL-12 was not efficiently delivered to the lymph nodes, we took biopsy specimens obtained from patients unrelated to the clinical trial and extracted the mononuclear cells from these specimens. The specimens were obtained from patients with lymphoma but also from normal lymphoid tissue. The cells were cultured in vitro with or without IL-12. The changes in gene expression were compared with the tumor biopsy specimens and normal peripheral blood cells obtained from patients in the study. The gene profiles of tumor cells in vivo and in vitro were similar and differed substantially from the gene profile of normal cells (see Fig. 4A). Due to the small number of biopsy specimens, the data need to be interpreted cautiously; however, these findings suggested that signaling through the IL-12 receptor may be insufficient to

![Fig. 3.](https://www.aacrjournals.org/clinican/images/clinican-vol12-no20-fig03.png)
activate intratumoral immune cells or that the intratumoral cells may require activation through an alternate pathway.

**Intratumoral T-cell repertoire after therapy with IL-12 and rituximab.** T-cell spectratyping results, where both tumor and peripheral blood specimens were evaluated pretreatment and posttreatment, were available for nine patients. Of these nine patients, two were assigned to arm B. One of the arm B patients had a complete response to rituximab alone and never

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**Fig. 4.** Changes in gene expression and T-cell repertoire after treatment with IL-12 and rituximab. A, RNA from peripheral blood mononuclear cells as well as lymph nodes involved by lymphoma was obtained from the same patient at baseline and after 2 weeks of therapy. Gene expression profiles were determined using the Affymetrix U133 Plus chip. Significant up-regulation of IFN-related genes was seen in the peripheral blood cells but not in the malignant cells in the involved lymph node. B, differences in the mean number of peaks and the mean proportion of peaks productively rearranged in the peripheral blood and in the malignant lymph node of nine patients before and after treatment. Patients 3 and 7 were from arm B; the remaining patients were from arm A. No distinct patterns or trends were observed.
received the IL-12. The other arm B patient had stable disease and went on to receive the IL-12, but no subsequent response was observed. Of the arm A patients, two had a response to treatment with both rituximab and IL-12 given simultaneously: one partial response and one complete response. Given the anticipated effects of IL-12 on the immune function of patients, we expected an increase in the mean number of peaks indicating more diversity of the T-cell receptor repertoire. In addition, we anticipated that the mean proportion of peaks that were productively rearranged would also increase, indicating a more functional immune system with productive T-cell receptors. An evaluation of changes in T-cell repertoire via these spectratype measures showed no distinct patterns or trends (see Fig. 4B), and no consistent finding suggesting optimization of the T-cell repertoire was seen with the addition of IL-12 to rituximab. Of potential interest is that there was greater variability in the changes in T-cell receptor repertoire measures for samples from the arm A patients who received concurrent rituximab and IL-12. Five of the seven arm A patients on whom we had tumor samples had an increased mean proportion of peaks productively rearranged; however, there did not seem to be any pattern related to clinical response.

**Discussion**

Rituximab is commonly used in the treatment of follicular and other low-grade NHLs (9–14). Although the antibody has been shown to directly induce apoptosis (15, 16), the Fc domain has also been shown to recruit immune effector functions to mediate lysis of the B cell (17). Recent studies have concluded that complement-mediated cytotoxicity and antibody-dependent cellular cytotoxicity are major mechanisms of action of rituximab in B-cell lymphomas (18, 19). In clinical studies, rituximab has been shown to induce objective responses in ~30% to 50% of patients treated (9–14, 20–24).

IL-12 as a single agent has been shown to have potent antitumoral activity in animal models, and objective tumor responses have been seen in patients with renal cell carcinoma, melanoma, and lymphoma (25–32). These clinical responses seemed to correlate with the induction of IFN-γ (26). This biological effect of IL-12 in up-regulating cell-mediated immunity was the reason for initially combining IL-12 with rituximab (7). The present study was, therefore, done to further determine the efficacy and toxicity of the combination of IL-12 and rituximab in patients with B-cell NHL.

Significant toxicities have previously been reported in the previous phase I and II studies of IL-12 as a single agent (29, 33). However, the combination of IL-12 and rituximab as given in this study was well tolerated with acceptable toxicity. There were, however, more grade 3 and 4 toxicities when IL-12 and rituximab were given together (arm A) than when they were given separately (arm B), and the toxicities were predominately associated with the administration of IL-12.

The concomitant use of IL-12 and rituximab had antitumor activity in patients with B-cell NHL treated in this study. Of the 30 evaluable patients accrued to arm A across all histologies, 11 responses (37%) were observed, and 13 responses (52%) were observed in the 25 patients on arm B. All responses observed in arm B were due to rituximab and none from subsequent treatment with IL-12. A confounding variable in the analysis of response is the fact that enrollment in the study was not limited to patients with follicular lymphoma alone. Patients with other indolent lymphomas that may be less responsive to rituximab, such as small lymphocytic lymphoma and MCL, were also included in the study. The lower response rate in arm A is, therefore, possibly due to a lower number of patients with follicular lymphoma in this cohort. A further factor that could account for the lower response rate in arm A is the fact that more patients in this group had previously received rituximab. Although patients who respond to rituximab may respond to retreatment, the response rate is lower than for those who have never received rituximab. Furthermore, a previous response to rituximab was not required for inclusion in the study, and this may have resulted in the lower response rate seen in arm A.

Despite this lack of additional activity in terms of clinical response when IL-12 was added to rituximab, the median time to progression on both arms was higher than that previously reported in the pivotal trial of rituximab alone (9). In the present study, the time to progression for responders on arm A was also longer than that observed in the pivotal trial: 19.1 (95% CI, 11.2, not reached) versus 12.5 months (95% CI, 11.0–16.0). Although this is not sufficient evidence to state that the time to progression for rituximab with concurrent IL-12 is significantly longer than rituximab alone, it does indicate that there may be some additional antitumor activity with the addition of IL-12 to rituximab.

An analysis of changes in gene expression and T-cell receptor repertoire when IL-12 was added showed that changes in the tumor differed from changes in the peripheral blood. Changes seen after exposure to IL-12 in vitro (in normal lymphoid tissue from patients not involved in the study) differed significantly from tumor specimens treated with IL-12 either in vitro (pretreatment and posttreatment biopsies from patients on the clinical trial) or in vitro (lymphoma biopsy specimens from patients not on study cultured in the presence or absence of IL-12). Tumor specimens showed none of the changes in gene expression expected to be induced by IL-12 and the i.t. T-cell repertoire remained unchanged after treatment. It is important to note, that due to the very small specimens obtained from patient biopsies, the results obtained from gene expression analysis array could not be verified with an alternative method, such as Western blot analysis or real-time PCR. However, these findings suggest that the i.t. immune cells may be inhibited by other factors in the tumor microenvironment, such as regulatory cells or other cytokines, or may require additional stimulation other than IL-12 via other mechanisms to be activated.

This finding is highly relevant as multiple clinical trials are in progress, combining rituximab with agents that stimulate the immune response, such as IL-2 and CpG-containing oligodeoxynucleotides. Based on the results of this study, it is possible that these strategies may not improve the response to rituximab, and it may be preferable to consider strategies that deplete cells that inhibit the immune response rather than attempting to stimulate immune cells that have been rendered inert by inhibitory factors. Recent data suggest that regulatory T cells are present in significant numbers in areas involved by B-cell lymphoma, and that these regulatory cells suppress the function of other infiltrating immune cells (34). We contend that future strategies using cytokines to stimulate the immune system.
response to the malignant B cells in NHL patients will first require the depletion of regulatory T cells to be effective. From the results of this randomized phase II trial, we concluded that the combination of IL-12 and rituximab was well tolerated with acceptable toxicity in patients with B-cell NHL. We found that the sequential administration of IL-12 at the time of disease progression or nonresponse after treatment with rituximab resulted in no additional clinical responses. In contrast, the concomitant use of IL-12 and rituximab had moderate disease activity in this study, but the response rate seen with IL-12 in combination with rituximab did not seem better than the results that would be expected in patients treated with rituximab alone. However, the concomitant administration of IL-12 did result in a prolonged time to disease progression. To determine whether the concomitant administration of IL-12 and rituximab is superior to rituximab alone will require a randomized phase III clinical trial.

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References

Clinical Cancer Research

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