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Lenalidomide and Rituximab for the Initial Treatment of Patients With Chronic Lymphocytic Leukemia: A Multicenter Clinical-Translational Study From the Chronic Lymphocytic Leukemia Research Consortium

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ABSTRACT

Purpose

Lenalidomide is an immunomodulatory agent with therapeutic activity in chronic lymphocytic leukemia (CLL). In preclinical models, lenalidomide acted synergistically with rituximab. The CLL Research Consortium initiated a phase II study to evaluate this combination in treatment-naive patients.

Patients and Methods

Lenalidomide was initiated at 2.5 mg/day and was escalated based on treatment tolerability to a maximum of 10 mg/day, for 21 days/cycle, for a maximum of seven cycles. Rituximab was administered at the end of cycle 1 and was continued for seven cycles. Patients received allopurinol and aspirin for prophylaxis.

Results

Sixty-nine patients enrolled onto one of two age-specific strata; patients' median age was 56 and 70 years for arms A and B, respectively. Patients in the older-patient stratum more frequently had elevated serum beta-2 microglobulin levels, high-risk Rai stage, and were less likely to complete the maximum planned therapy. Adverse events were similar in the two arms. Nonhematologic toxicity was predominantly at grade 1/2, and neutropenia was the most common hematologic adverse event. The response rate for arm A was 95%, with 20% complete responses (CRs) and 20% nodular partial responses. Of arm B patients, 78% achieved a response, of which 11% were CRs. Median progression-free survival (PFS) was 19 months for the younger cohort and 20 months for the older cohort.

Conclusion

Intrapatient dose-escalation was safe. The majority of patients reached the maximum lenalidomide dose and experienced a response to a defined seven-cycle course of lenalidomide and rituximab therapy. Despite differences in baseline characteristics and the response rate between the two strata, the PFS did not differ.

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INTRODUCTION

Lenalidomide (Revlimid; Celgene, Summit, NJ) is an immunomodulatory compound that was approved for multiple myeloma and myelodysplastic syndrome. Lenalidomide is active when administered to patients with chronic lymphocytic leukemia (CLL).[1-4] Reports of single-agent lenalidomide in patients with relapsed CLL detailed overall response rates (ORR) of 32% to 58% and a tumor-flare reaction (TFR) in more than half the patients.[1,2] Initial starting doses of 25 mg were associated with tumor lysis syndrome (TLS), mandating that trials initiate treatment at doses of 10 mg.[2,5,6] Subsequently, TLS observed in treatment-naive patients prompted a more conservative approach, beginning with doses of 2.5 mg.[3] Investigators from The University of Texas MD Anderson Cancer Center reported on lenalidomide as initial therapy at 5 mg in 60 older patients with CLL.[1] Lenalidomide monotherapy in this population was associated with a 65% ORR, including 10% complete responses (CRs).

The immune modulatory effects of lenalidomide may account for its clinical activity in CLL.

Upregulation of costimulatory molecules on leukemia cells after in vitro exposure to lenalidomide lead to an activation phenotype that is similar to that induced in CD154 gene therapy studies.[7-9] Lenalidomide has reversed deficits in leukemia patients' cognate immune cell interactions, improving the capacity of CLL cells to form immune synapses with T cells.[8,9] Lenalidomide treatment is associated with expansion of immune effector cells and enhanced antibody-dependent cellular cytotoxicity to rituximab in mouse lymphoma models.[10,11] These data provide a rationale for coadministration of this immunomodulatory compound with monoclonal antibodies. However, published data indicating lenalidomide could reduce CD20 expression in vitro has raised concern that lenalidomide could mitigate the activity of rituximab.[12]

Rituximab is an anti-CD20 monoclonal antibody that is approved for the treatment of patients with CLL in combination with chemotherapy.[13] Rituximab, when administered as front-line treatment with fludarabine and cyclophosphamide, significantly improved the outcomes of CLL patients.[14,15] However, despite these advances, intensive chemoimmunotherapy regimens are not well tolerated by older patients or those with comorbidities.[16] Novel treatment approaches for treatment-naive CLL patients are still needed.

Immune therapy represents a promising approach in CLL as demonstrated by the improved outcomes of patients treated with chemoimmunotherapy and long-term disease control with allogeneic hematopoietic stem-cell transplantation. The immune-based treatment combination of lenalidomide and rituximab in relapsed-CLL patients was associated with an ORR of 66% with 12% CRs; these deeper responses were achieved after 12 months of therapy.[17] Rituximab was administered before lenalidomide, a sequence that may have decreased the rate of TFR, based on comparisons of single-agent lenalidomide from the same institution.[4,17]

Anti-CD20 therapy plays an important role in CLL. The CLL Research Consortium (CRC) want to develop chemotherapy-free immunotherapeutic approaches and initiated a phase II study of two parallel age-specific strata to evaluate the combination of lenalidomide and rituximab as initial treatment. A low starting dose allowing for intrapatient dose escalation was chosen, owing to toxicity observed in an ongoing treatment-naive study.[3,6] Lenalidomide was initiated in a lead-in design before rituximab, providing an opportunity to evaluate changes in leukemia-cell phenotype induced by lenalidomide.

PATIENTS AND METHODS

Patient Eligibility

Patients were required to be diagnosed with CLL and be treatment-naive, with indication for treatment based on the International Workshop for CLL guidelines (iwCLL).[18] Patients needed an Eastern Cooperative Oncology Group performance status of 2 or lower, serum creatinine levels of less than 1.5 mg/dL, and normal hepatic function. Patients with Hepatitis B or C or HIV infection, recent myocardial infarction, or stroke were excluded. Patients with a history of deep vein thrombosis/pulmonary embolus were also excluded because of the association of lenalidomide and thromboembolism. All patients provided informed consent. Research was approved by local human research protections programs and institutional review boards, and studies were conducted in accordance with the Declaration of Helsinki.

Study Design and Treatment

Treatment-naive CLL patients were enrolled onto one of two age-specific cohorts: patients younger than 65 years and those 65 years old and older. All patients began lenalidomide treatment at 2.5 mg daily for days 1-7 of the first cycle and were able to escalate to 5 mg daily for days 8-21 of the first cycle. Patients continued at 5 mg daily for the second cycle and were able to escalate to a maximum of 10 mg daily for cycles three through seven, provided there were no toxicities of grade 2 or higher. Lenalidomide was administered for 21 days, followed by a period of rest each cycle, for a maximum of seven cycles. Cycle 1 was 35 days long, and cycles 2 to 7 were 28 days long. To allow for tumor cell sampling, the first dose of rituximab was initiated on day 29 of cycle 1. After a test dose of 50 mg/m^2 of rituximab, an additional 325 mg/m^2 was administered on day 31, and yet another dose of 375 mg/m^2 on day 33. Rituximab was then continued at 375 mg/m^2 weekly throughout cycle 2 in a dose-dense fashion and then was administered on day 1 of cycle 3 to 7. Before the start of lenalidomide and any dose escalation, patients received allopurinol, oral hydration, and underwent laboratory monitoring for TLS.

Dose reductions were mandated for toxicity. Treatment was held for toxicity levels that were grade 3 or higher for neutropenia, febrile neutropenia, or thrombocytopenia, and treatment was resumed at lowered doses when toxicity resolved. Neutrophil growth factors were administered per American Society of Clinical Oncology guidelines.[19] For nonhematologic toxicities that

Table 1. Baseline Characteristics by Strata

Characteristic	Arm A (< 65 years old; n = 40)		Arm B (≥ 65 years old; n = 29)		
	No. of Patients	%	No. of Patients	%	P
Age, years					
Median	57		70		
Range	45-64		65-80		
ECOG PS*					.01
0	29	73	10	35	
1 or 2	11	28	17	59	
Rai stage					.07
≤ II	30	75	15	52	
III-IV	10	25	14	48	
B$_2$M, mg/L†					.01
≤ 3.5	28	70	11	38	
> 3.5	10	25	16	55	
IGHV mutation‡					.33
Mutated	15	38	14	48	
Not mutated	25	63	14	48	
ZAP-70					.31
Negative	27	68	17	59	
Positive	13	33	12	41	
CD38					.23
Negative	24	60	13	45	
Positive	16	40	16	55	
FISH/cytogenetics					.94
13q del	14	35	11	38	
Normal	7	18	5	17	
Trisomy 12	10	25	6	21	
11q del	5	13	4	14	
17p del	4	10	2	7	
Other, t(18,22)	0	0	1	3	
CIRS ≥ 6	NA		10	35	
Range			0-11		

Abbreviations: B$_2$M, β_2-microglobulin; CIRS, cumulative illness rating score; del, deletion; ECOG PS, Eastern Cooperative Oncology Group performance status; FISH, fluorescent in situ hybridization; IGHV, immunoglobulin heavy-chain variable-region genes; NA, not available; ZAP-70, 70kDa zeta-chain-associated protein.
*ECOG PS is missing for two patients in arm B.
†B$_2$M is missing for two patients on each arm.
‡IGHV mutation result of one patient in arm B is missing.

Table 2. Common Adverse Events by Strata

Adverse Event	Arm A (< 65 years old; n = 40)			Arm B (≥ 65 years old; n = 29)		
	Grade I/II (No. of patients)	Grade III/IV (No. of patients)	Any Grade (%)	Grade I/II (No. of patients)	Grade III/IV (No. of patients)	Any Grade (%)
Neutropenia	9	21	75	3	19	76
Tumor flare reaction	33	0	83	18	1	66
Fatigue	29	2	78	18	2	69
Anemia	20	4	60	18	3	72
Transaminases	22	3	63	13	4	59
Rash	21	2	58	17	2	66
Thrombocytopenia	23	1	60	16	1	59
Infusion reaction	28	0	70	6	3	31
Creatinine	20	0	50	10	0	35

were grade 3 or higher, treatment was withheld until the event resolved to grade 2 or lower; then, treatment was resumed at a reduced dose. Patients who were receiving 2.5 mg and experienced such toxicity discontinued lenalidomide therapy. Cardiac arrhythmias of grade 3 or higher or desquamating rash required drug discontinuation. Glucocorticoids were used to mitigate TFR, but not as prophylaxis. Methylprednisolone (Medrol DosePak; Pfizer, New York, NY) was used in a minority of patients with TFR. After two pulmonary emboli were observed in the first 31 patients, the protocol was amended to mandate thromboprophylaxis with acetylsalicyclic acid 81 mg daily.

The primary objective was to determine the CR rate for each strata. Secondary objectives were to evaluate the safety of the combination, determine ORR, progression-free survival (PFS), overall survival, and the incidence of TFR. Exploratory end points evaluated the impact of in vivo lenalidomide on the immune phenotype of leukemia cells and the relationship between Fc receptor (FcR) polymorphisms and response to therapy. Clinical activity was evaluated by iwCLL response criteria with multiparameter flow cytometry to detect minimal residual disease in the marrow.[18,20]

Assessments

Patients underwent baseline assessment for toxicity on days 8 and 29 of cycle 1, weekly during cycle 2, and on the first day of subsequent cycles. Full response evaluations were performed at 2 months and later following treatment completion. Subsequently, patients completed follow-up examinations every 3 months for the first year and every 6 months thereafter. Cumulative illness rating scale[21] scoring was performed on older patients.

Criteria for Response and Toxicity

Response was determined according to the iwCLL. Toxicity was reported according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 4, for nonhematologic toxicities and according to the iwCLL[18] for hematologic toxicities. TFR was characterized by enlarged and tender lymph nodes or spleen.

Biologic Prognostic Markers

Analysis of genomic aberrations by interphase fluorescent in situ hybridization, immunoglobulin heavy-chain variable-region genes (IGHV) mutational status, and expression of CD38 and ZAP-70 by flow cytometry were provided by the CRC Tissue Core.

Immune Phenotype Assessment

Blood samples were obtained from consenting patients at baseline and day 22, before rituximab. Mononuclear cells were isolated using density-gradient centrifugation. Cells were viably cryopreserved in 10% dimethyl sulfoxide and 90% fetal-calf serum. CLL cells were immunophenotyped by flow cytometry analyzed for CD19, CD20, DR5 (CD262), CD95, HLA-DR, CD54, CD80, CD86, CXCR4, and CXCR5, with the use of monoclonal antibodies conjugated to allophycocyanin, peridinin-chlorophyll A-protein complex, or fluorescein isothiocyanate. Fluorochrome-conjugated, isotype control monoclonal antibodies of irrelevant specificity were used in all experiments to monitor for nonspecific staining. The absolute mean fluorescent intensity was determined on CD19+ lymphocytes by subtracting the mean fluorescent intensity of isotype-control stained cells. Absolute changes of each protein were assessed for association with response and TFR.

Assessment of Fc Gamma Receptor Polymorphisms

DNA was extracted from viably frozen mononuclear cells using the QIAamp kit (Qiagen, Valencia, CA), according to manufacturer's instructions. A nested polymerase chain reaction strategy was performed to assess for allelic isoforms of FcγR IIIa or IIa.[22]

Statistical Methods

Patient characteristics are summarized as numbers and percentages for categoric variables and medians and ranges for continuous variables. The cutoff points for IGHV mutation, ZAP-70, and CD38 were 98%, 20%, and 30%, respectively.[23,24] Final response is summarized as numbers and percentages with 90% binomial CIs. Associations between patient characteristics and response were assessed using Fisher's exact tests. PFS is defined as the time from start of treatment to date of disease progression or patient death, or was censored on the date that patients without disease progression were last known to be alive. A Kaplan and Meier estimate was used to summarize median PFS. Expression values of each immune-phenotypic marker at pre- and post-treatment and the difference between the two are summarized as medians and interquartile ranges. Wilcoxon's signed rank test was used to assess whether the expression level of a particular marker on the leukemia cells significantly changes after starting treatment.

Table 3. Final iwCLL Response to Therapy by Strata

Final iwCLL Response to Therapy	Arm A (< 65 years old; n = 40)		Arm B (≥ 65 years old; n = 29)	
	No. of Patients	%	No. of Patients	%
Complete response	8	20	2	7
CRi	0		1	3
Nodular partial response	7	18	0	
Partial response	23	58	20	69
Stable disease	0		3	10
Progressive disease	0		1	3
Nonevaluable	2	5	2	7
Overall response rate	38	95	23	79
95% CI	85 to 99		63 to 91	
Complete response, including CRi	8	20	3	10
95% CI	10 to 33		3 to 25	

Abbreviations: CRi, complete response incomplete marrow recovery; iwCLL, International Workshop for Chronic Lymphocytic Leukemia.

RESULTS

Patients Characteristics

Patient characteristics are listed in Table 1. Sixty-nine patients, with a median age of 62 years, enrolled onto one of two age-specific strata: arm A (< 65 years old) and arm B (≥ 65 years old). The median age in arm A was 57 years and in arm B was 70 years. In arm B, the patients had compromised performance status and elevated serum β-2 microglobulin levels. Older patients had a numerically higher incidence of high-risk Rai stage that was not significant.

Therapy

In arm A, 40% of patients (16 of 40) were able to escalate their treatment doses when first permitted, per maximal protocol allowance, compared with only 21% of patients (six of 29) in arm B ($P = .12$). Most patients were able to escalate their doses at some point during study, providing a median dose of lenalidomide for patients in either strata of the maximal daily dose allowed (10 mg). However, patients in arm B were less likely than patients in arm A to complete all seven cycles of therapy (59% [17 of 29] v 88% [35 of 40], respectively; $P = .01$).

Toxicity

The most commonly observed adverse events were similar between the two arms (Table 2). Neutropenia was observed frequently and tended to be severe. Anemia and thrombocytopenia were frequently grade 1/2. TFR was mild-to-moderate in severity, except for that observed in one patient in arm B. TFR was predominantly observed during cycle 1, with the incidence decreasing following administration of rituximab. Only one patient had first occurrence of TFR after cycle 1. Hepatic transaminase elevations were observed frequently early in therapy with lenalidomide and were generally resolved with continued treatment. No thromboembolism occurred after aspirin was mandated for thromboprophylaxis. The incidence of Grade ≥ 3 toxicity was similar in the two arms at 70% and 83%, respectively, with severe nonhematologic toxicities occurring in 40% and 62% of patients, respectively (Appendix Table A1 [online-only]). A cumulative illness rating scale score of 6 or higher was not associated with a higher frequency of grade ≥ 3 toxicity.
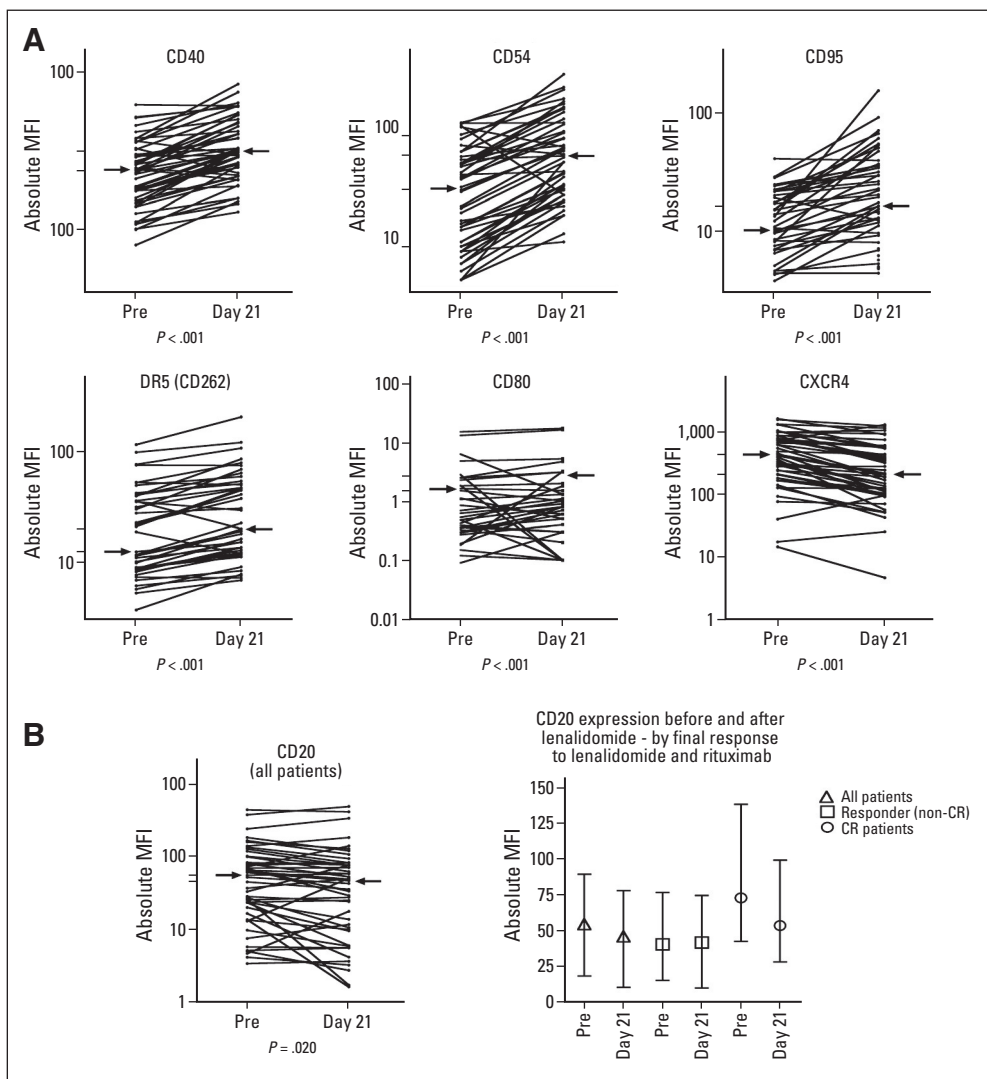
Fig 1. (A) In vivo modulation of chronic lymphocytic leukemia (CLL) surface proteins with exposure to single-agent lenalidomide absolute mean fluorescent intensity (MFI) of protein for each patient's CD19+ cells before and after 21 days of single-agent lenalidomide. Median MFI levels for pre- and post-treatment are denoted by arrows on each respective side. (B) CLL surface protein expression of CD20 before and after single-agent lenalidomide and by final International Workshop for Chronic Lymphocytic Leukemia (iwCLL) response to combination therapy with lenalidomide and rituximab absolute MFI of protein for each patient's CD19+ cells before and after 21 days of single agent lenalidomide. (Left panel) Median MFI values for pre- and post-treatment are denoted by arrows on each respective side. (Right panel) Before (pre) and after day 21 single-agent lenalidomide absolute MFI of CD20, medians with interquartile range are displayed based on final iwCLL response to combination therapy with lenalidomide and rituximab. (+) cells before and after 21 days of single-agent lenalidomide. CR, complete response.

Response to Therapy

The ORR in arm A was 95%, with 20% achieving a CR and 20% a nodular partial response (nPR; Table 3). The ORR in arm B was 79%, with 10% of patients achieving a CR, including one CR with incomplete hematologic recovery.[25] One patient in each strata achieved a minimal residual disease–negative CR (Appendix Table A2). The ORR for both strata combined was 88%, with 15% achieving CR and 12% nPR. Appendix Table A2 lists patient characteristics and their association with response to therapy. Four of six patients with CLL who had del(17p) and eight of nine patients with del(11q) responded to therapy.

FcR Polymorphism

Evaluation for polymorphism in FcR did not reveal an association between certain alleles of FcR IIIa and IIa, as assessed by single-nucleotide polymorphism analyses, and response (Appendix Table A3).

Leukemia-Specific Immune-Phenotypic Changes

We observed relative increases in leukemia cell expression of immune costimulatory proteins (CD40, CD54, and CD86) in HLA-DR and death receptors (CD95 and DR5) during the first 21 days of single-agent lenalidomide administration (Fig 1; Appendix Table A4). In contrast, lenalidomide treatment caused significant reduction in leukemia-cell expression of chemokine receptors CXCR4 and CXCR5.

The levels of CD20 expressed by CLL cells were either unchanged or decreased after 21 days of lenalidomide treatment. Collectively, there was a modest decline in the level of CD20 that was of borderline significance. Nevertheless, a drop in CD20 expression with lenalidomide did not preclude patients from achieving a CR in response to lenalidomide and rituximab. The CLL cells of patients who achieved a CR often had a larger decrease in CD20 expression ($P = .07$; Fig 1B). CLL cells of patients who achieved a CR tended to have higher expression levels of CD20 before and 21 days after the initiation of lenalidomide than did the CLL cells of patients with poorer quality responses; however, this difference was not statistically significant. We noted changes in a number of parameters including a significant increase in the expression level of CD54 observed in leukemia cells of patients who experienced TFR compared with those who did not ($P = .02$; Appendix Table A5).

Follow-Up

Over a median follow-up period of more than 20 months we observed a median PFS of 19 months in patients in arm A and 20 months in arm B (Fig 2). We investigated for an association between degree of response and PFS among responders from time of response assessment. Patients who achieved a CR had a significantly longer median PFS (21 months) than that of patients who achieved a partial response or nPR (9 months; $P = .008$; Fig 3). Median overall survival has not been reached in either arm. One patient died in arm A and four died in arm B. No patients died within 30 days of study treatment.

DISCUSSION

Immune therapy offers a promising approach to the treatment of patients with CLL. To examine a chemotherapy-free immune therapy
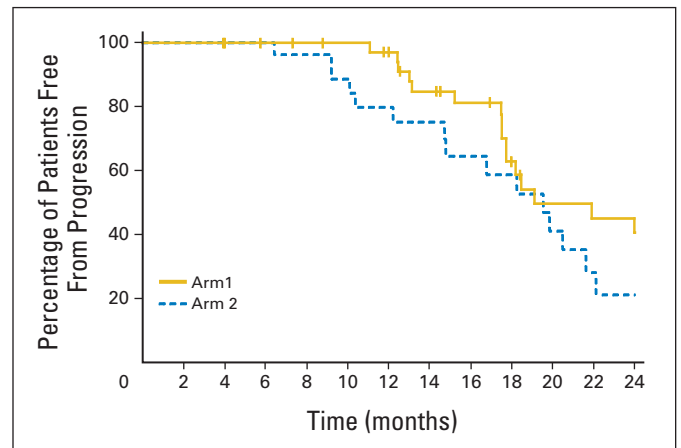
Fig 2. Progression-free survival (PFS) curves stratified by patients younger than 65 years or 65 years or older. Kaplan-Meier PFS curve for the age-specific strata with a median follow-up of 20 months for both arms. In arm 1, the 40 patients younger than 65 years, represented by a solid gold line, experienced 19 events with median PFS estimated at 19 months. In arm 2, the 29 patients age 65 years or older, represented by a blue dashed line, experienced 17 events with a median PFS estimated at 20 months.

regimen for CLL, the CRC designed a phase II study evaluating seven cycles of lenalidomide and rituximab that were administered to two independent age-specific strata of treatment-naive patients. The lead-in design allowed for an in vivo evaluation of the impact of single-agent lenalidomide on the immune phenotype of leukemia cells. Special attention was paid to changes in CLL-cell expression of CD20 during the first cycle, because changes in CD20 could influence the efficacy of subsequent or concomitant therapy with rituximab.[12] A previous study reported that lenalidomide could downregulate expression of CD20 on CLL cells treated in vitro.[12] However, we only observed modest changes in the expression levels of CD20 after 21 days of lenalidomide monotherapy; whereas the CLL cells of some patients had lower levels of CD20, the cells of others had no detectable
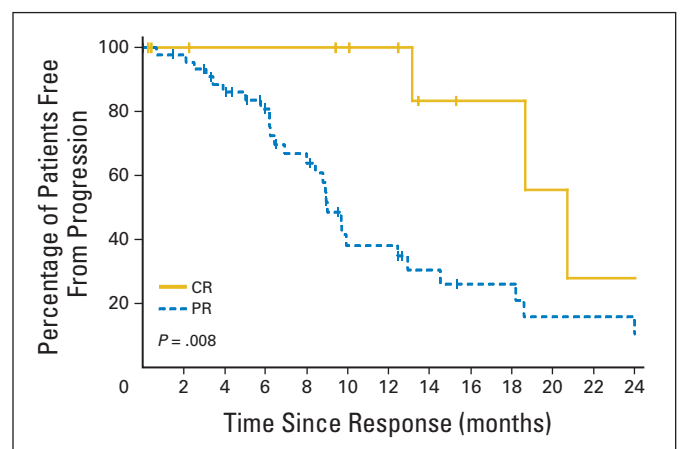
Fig 3. Progression-free survival (PFS) curves by final response to therapy. Kaplan-Meier PFS curve by the final response to therapy for all patients enrolled. Of the 11 patients who achieved a complete response (CR), represented by a solid gold line, four experienced PFS events with median PFS from time of response estimated at 21 months. Of the 44 patients who achieved a partial response (PR) or nodular partial response (nPR), represented by a blue dashed line, 29 experienced PFS events with a median PFS estimated at 9 months from time of response. PFS of CR patients was longer than PR/nPR patients; $P = .008$.

change. We did not observe a significant association between the magnitude of change in the expression levels of these proteins and response to therapy. This might be because of the high frequency of responders (88%) and the small sample size of nonresponders. Nevertheless, it is noteworthy that patients who had CLL cells that had reduced expression of CD20 during lenalidomide therapy were not precluded from achieving a CR in response to lenalidomide and rituximab. The lenalidomide lead-in provided an opportunity to evaluate CD20 expression, which could not be appreciated if rituximab was administered before lenalidomide. The modest impact of lenalidomide on CD20 and the ability of the combination to induce CRs argue against the notion that these agents should not be used in combination.

The leukemia cell-specific changes in the expression of costimulatory molecules and chemokine receptors were examined in context of the TFR. This revealed an association between the relative upregulation of CD54 on CLL cells of patients who experienced TFR. However, the functional significance of this relationship or association with celebron levels, the only identified target of lenalidomide, is not known.

The baseline characteristics between strata were different, with patients 65 years old and older more likely to have elevated serum $\beta2$-microglobulin levels ($P = .01$), Eastern Cooperative Oncology Group performance status of at least 1 ($P = .05$), or high-risk Rai-stage disease ($P = .07$). Molecular and genetic factors were similar between the two strata, with the CLL cells of the enrolled population often exhibiting high-risk features, such as use of unmutated IGHV or having del(17p) or del(11q).

Intrapatient dose-escalation schema was safe, with the majority of patients reaching the maximum dose allowed for this study. The overall adverse event profile, including the incidence of grade ≥ 3 toxicities, was similar between these two strata. Neutropenia was the most common hematologic adverse event. Lenalidomide treatment has been reported to increase serum immunoglobulin levels,[4] however, this was not noted in a subset of patients treated with the rituximab combination in our current study. Eighty-three percent and 66% of patients in the older- or younger-patient cohorts, respectively, experienced TFR of about grade 1/2 in severity. TFR itself was not significantly associated with achieving a response to therapy as has previously been reported.[2] However, as in the current study, TFR may limit the dose-escalation of lenalidomide. This assessment may be confounded by the maximum-achieved dose. Importantly, the definition of TFR did not require coinciding fevers or rash as detailed in earlier reports.[2] Younger patients tended to experience TFR and infusion reactions more frequently. Older patients were less likely to escalate rapidly, maintain the maximum dose of lenalidomide, and were more likely to discontinue treatment early. Recently, a phase III trial of lenalidomide versus chlorambucil in older patients with treatment-naive CLL was terminated owing to a higher number of deaths in the lenalidomide arm. Although the combination of lenalidomide and rituximab is active, the risks must be considered.

Compared with other reported studies in which lenalidomide is typically given until disease progression or toxicity, our current study evaluated the clinical activity of a defined course of seven cycles of therapy, although longer duration of therapy might prolong PFS while patients are on therapy. Nevertheless, this regimen was highly active; almost all patients (88%) achieved a response to therapy. Responses were achieved in patients who had CLL cells with high-risk features, such as del(17p) (67%) or del(11q) (89%), confirming previous reports.[4,26]The observed response rate with lenalidomide and rituximab in patients with del(17p) is on par with the ORR of patients with del(17p) who received initial therapy with fludarabine and cyclophosphamide (68%).[15] Younger patients experienced a higher ORR, including more CRs and nPRs, than did patients in the older-patient strata. Despite differences in pretreatment characteristics and response to therapy, median PFS associated with this seven-cycle course of therapy was similar between the two strata (19 v 20 months, respectively). Lenalidomide and rituximab represents a highly active immune-based therapy approach for treatment-naive CLL patients, in particular those with high-risk cytogenetics.

AUTHOR CONTRIBUTIONS

Conception and design: Danelle F. James, Donna Neuberg, Thomas J. Kipps
Provision of study materials or patients: Jennifer R. Brown
Collection and assembly of data: Danelle F. James, Lillian Werner, Jennifer R. Brown, William G. Wierda, Jacqueline C. Barrientos, Januario E. Castro, Andrew Greaves, Laura Z. Rassenti, Kanti R. Rai, Thomas J. Kipps
Data analysis and interpretation: Danelle F. James, Lillian Werner, Jennifer R. Brown, Januario E. Castro, Amy J. Johnson, Donna Neuberg, Thomas J. Kipps
Manuscript writing: All authors
Final approval of manuscript: All authors

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Appendix

	Arm A (< 65 years old; n = 40)			Arm B (≥65 years; n = 29)			
AEs	Grade 3	Grade 4	Total	Grade 3	Grade 4	Total	Total (N = 69)
Neutropenia	13	8	21	8	11	19	40
Anemia	4	0	4	3	0	3	7
Pneumonia	1	0	1	6	0	6	7
Transaminases	3	0	3	4	0	4	7
Fatigue	2	0	2	1	1	2	4
Rash	2	0	2	2	0	2	4
Hyponatremia	2	0	2	1	0	1	3
Hypotension	0	0	0	3	0	3	3
Basal cell carcinoma	0	0	0	2	0	2	2
Fever	1	0	1	1	0	1	2
Hypophosphatemia	2	0	2	0	0	0	2
Infusion reaction	0	0	0	2	0	2	2
Pulmonary embolism	0	0	0	2	0	0	2
Thrombocytopenia	0	1	1	0	1	1	2
Allergic reaction	0	0	0	1	0	1	1
Anorexia	0	0	0	1	0	1	1
Becteremia	0	0	0	1	0	0	1
Cellulitis	0	0	0	1	0	0	1
Deep vein thrombosis	0	0	0	1	0	0	1
Diarrhea	1	0	1	0	0	0	1
Dyspnea	0	0	0	1	0	1	1
Edema	0	0	0	1	0	1	1
Headache and aseptic	1	0	1	0	0	0	1
Hemorrhage	0	0	0	1	0	1	1
Hyperbilirubinemia	0	0	0	1	0	1	1
Hyperglycemia	1	0	1	0	0	0	1
Hyperphosaphatemia	0	0	0	1	0	1	1
Hypersensitivity	1	0	1	0	0	0	1
Hypoalbuminemia	0	0	0	1	0	1	1
Hypoglycemia	0	0	0	0	1	1	1
Hypokalemia	1	0	1	0	0	0	1
Infection, lung	0	0	0	1	0	1	1
Infection, skin	0	0	0	1	0	1	1
Nausea	1	0	1	0	0	0	1
Neuropathy	1	0	1	0	0	0	1
Musculoskeletal	1	0	1	0	0	0	1
Myalgia	1	0	1	1	0	1	1
Myocardial infarction	0	0	0	1	0	0	1
Pain, abdominal	1	0	1	0	0	0	1
Pain, chest	0	0	0	1	0	1	1
Pain, knee	1	0	1	0	0	0	1
Pain, muscle	0	0	0	1	0	1	1
Post polio syndrome	1	0	1	0	0	0	1
Renal failure	0	0	0	1	0	1	1
Rigors/chills	0	0	0	1	0	1	1
Squamous cell carcinoma	0	0	0	1	0	1	1
Supraventricular tachycardia	1	0	1	0	0	0	1
Syncope	0	0	0	1	0	0	1

Table A1. All Grade 3 and Grade 4 AEs

(continued on following page)

Table A1. All Grade 3 and Grade 4 AEs (continued)

	Arm A (< 65 years old; n = 40)			Arm B (≥ 65 years; n = 29)			
AEs	Grade 3	Grade 4	Total	Grade 3	Grade 4	Total	Total (N = 69)
Tumor flare	0	0	0	1	0	1	1
Tumor lysis syndrome	0	0	0	1	0	1	1
Urinary retention	1	0	1	0	0	0	1
Urothelial carcinoma	1	0	1	0	0	0	1
Urticaria	0	0	0	1	0	1	1
Vomiting	1	0	1	0	0	0	1

Abbreviation: AE, adverse event.

Table A2. Response by Patient Characteristic by Strata

	Arm A (< 65 years old; n = 40)							Arm B (≥ 65 years; n = 29)						
		CR			ORR				CR			ORR		
Characteristic	Total No. of Patients	No. of Patients	%	P	No. of Patients	%	P	Total No. of Patients	No. of Patients	%	P	No. of Patients	%	P
All patients	40	8	20		38	95		29	3	10		23	79	
ECOG PS score*														
0	29	4	14	0.18	29	100	0.07	11	1	9	1.0	9	82	1.0
1-2	11	4	36		9	82		17	2	12		13	77	
Rai stage														
0-2	30	6	20	1	29	97	0.44	15	1	7	0.6	13	87	0.39
3-4	10	2	20		9	90		14	2	14		10	71	
B₂M mg/L†														
≤ 3.5	28	8	29	0.08	27	96	0.46	11	2	18	0.16	9	80	1.0
> 3.5	10	0	0		9	90		16				12	73	
IGHV mutation‡														
Mutated	15	3	20	1.0	14	93	1.0	14	1	7	1.0	11	79	1.0
Unmutated	25	5	20		24	96		14	1	7		12	86	
ZAP-70														1.0
Negative	27	7	26	0.24	26	96	1.0	17	2	12	1.0	13	77	
Positive	13	1	8		12	92		12	1	6		10	83	
CD38														
Negative	24	5	24	1.0	23	96	1.0	13	2	15	0.57	11	85	0.66
Positive	16	3	19		15	94		16	1	6		12	75	
FISH														
13q del	14	3	21	0.91	14	100	0.10	11	1	9	1.0	10	91	0.27
Normal	7	1	14		6	86		5	1	20		4	80	
Trisomy 12	10	3	30		10	100		6	1	17		4	67	
11q del	5	1	20		5	100		4	0			3	75	
17p del	4	0	0		3	75		2	0			1	50	
Len median dose, mg														
2.5	2	0	0	0.14	1	50	0.05	6	0		0.40	5	83	0.40
5.0	10	0	0		10	100		8	0			5	63	
10.0	27	8	30		27	100		15	3	20		13	87	
No. of cycles														
< 7	5	1	20	NA	3	60	NA	12	0		NA	7	58	NA
7	35	7	20		35	100		17	3	18		16	94	
Tumor flare														
No	7	2	40	0.61	7	100	1.0	12	3	25	0.06	10	83	1.0
Yes	33	6	18		6	86		17	0			13	77	

Abbreviations: B₂M, β2-microglobulin; CR, complete response; del, deletion; ECOG PS, Eastern Cooperative Oncology Group performance status; FISH, fluorescent in situ hybridization; IGHV, immunoglobulin heavy-chain variable-region genes; Len, lenalidomide; NA, not available; ORR, overall response rate; ZAP-70, 70kDa-zeta chain-associated protein.
*ECOG PS is missing in two patients in arm B.
†B₂M missing in two patients on each arm.
‡IGHV mutation result of one patient in arm B is missing.

Table A3. Association of Fc Gamma Receptor SNP and Final Response to Lenalidomide and Rituximab

Patient Subgroup	Total No. of Patients	CR No. of Patients	%	P	ORR No. of Patients	%	P
All patients	69	11	16		61	88	
Fc gamma IIa				.44			1.00
H	10	1	10		9	90	
R	14	4	29		12	86	
RH	36	5	14		31	86	
Fc gamma IIIa				1.00			.86
F	22	4	18		19	86	
FV	33	5	15		29	88	
V	5	1	20		4	80	

Abbreviations: CR, complete response; SNP, single-nucleotide polymorphism; ORR, overall response rate.

Table A4. Summary of MFI Expression Before and After Treatment With 21 Days of Single-Agent Lenalidomide and the Change in Absolute MFI Association With Achieving a CR

Antigen	No. of Patients	Absolute MFI Before Treatment Median	IQR	Absolute MFI After Treatment Median	IQR	Absolute MFI Change Median	IQR	P	Absolute MFI Change CR (yes; n = 8) Median	IQR	Absolute MFI Change CR (no) Median	IQR	P
CD20	49	54.15	19.65-81.00	45.60	10.40-74.90	−6.5	−25.9-2.19	.02	−21.49	−61.2-2.4	−4.18	−57.0-109.2	.07
CD40	49	23.60	15.80-29.60	31.19	22.58-46.46	8.30	4.90-16.20	< .001	8.25	2.8-17.6	8.25	−9.7-44.7	.98
CD54	50	33.00	11.00-60.00	66.00	32.00-129.00	31.50	15.00-83.00	< .001	23.5	2.0-101.0	33.0	−93.0-281.0	.84
CD80	50	0.36	0.09-1.51	0.40	−0.10-1.00	0.14	−0.33-0.40	.45	0.31	−2.9-2.23	0.13	−0.5-3.27	.49
CD86	49	1.57	0.70-3.31	2.70	1.20-5.60	0.71	0.21-1.50	< .001	0.57	−0.33-5.50	0.71	−3.80-10.49	.79
CD95	49	10.20	4.60-17.60	16.30	8.00-35.50	6.31	2.56-12.30	< .001	11.10	−0.3-38.2	4.87	−9.91-139.9	.21
CXCR4	49	425.16	205.33-756.31	205.20	101.17-530.40	−167.90	−331.00-−51.70	< .001	−286.9	−472.1-−44.2	−148.8	−717.1-549.5	.25
CXCR5	49	413.30	268.70-621.20	261.20	189.90-422.00	−106.30	−252.10-−14.00	< .001	−108.45	−407.2-106.5	−104.85	−461.6-146.3	.91
DR5	45	12.43	8.68-35.91	20.00	12.10-50.10	5.16	2.60-14.63	< .001	4.76	1.49-22.8	3.6	−15.6-90.8	.93
HLADR	45	189.00	143.00-332.00	257.78	166.94-428.00	61.60	−6.30-166.20	< .001	557.5	−99.2-269.3	67.3	−269.6-457.0	.72

NOTE. Expression values of each marker before and after treatment as well as the change are summarized as medians and IQRs. Wilcoxon's signed rank test is used to assess whether the expression value for each marker differed before and after treatment, to assess the associations of changes in protein expression, and to assess whether patients achieved an overall response rate (data not shown) or CR.
Abbreviations: CR, complete response; IQR, interquartile range, MFI, mean fluorescent intensity.

Table A5. Change in Absolute MFI After Treatment With 21 Days of Single-Agent Lenalidomide and Association With Tumor Flare Reaction

Antigen	Absolute MFI Change (without TFR) Median	IQR	Absolute MFI Change (with TFR) Median	IQR	P
CD20	−3.76	−61.2-66	−7.9	−57-109.2	.91
CD40	5.15	−2.4-27.3	8.3	−9.7-44.7	.17
CD54	18	−14-281	42.5	−93-165	.02
CD80	−0.02	−2.9-0.4	0.23	−5-3.27	.23
CD86	0.41	−.38-5.9	0.78	−1.17-10.49	.28
CD95	4.34	−1.03-139.9	8.61	−9.91-62.4	.23
CXCR4	−78.45	−292.9-−4.2	−183.5	717-549.5	.13
CXCR5	−63.8	−285-146.1	−127.05	−461.6-146.1	.22
DR5	3.55	−5.7-18.17	7.1	−15.6-90.8	.10
HLADR	17.8	−117.7-282.4	106.6	269.6-457	.18

NOTE. Expression values of each marker at pre- and post-treatment, as well as the change, are summarized as medians and IQRs. Wilcoxon's signed rank test is used to assess whether the expression value for each marker differed pre- and post-treatment and were used to assess the associations of changes in protein expression and whether patients experienced TFR.
Abbreviations: IQR, interquartile range; MFI, mean fluorescent intensity; TFR, tumor flare reaction.
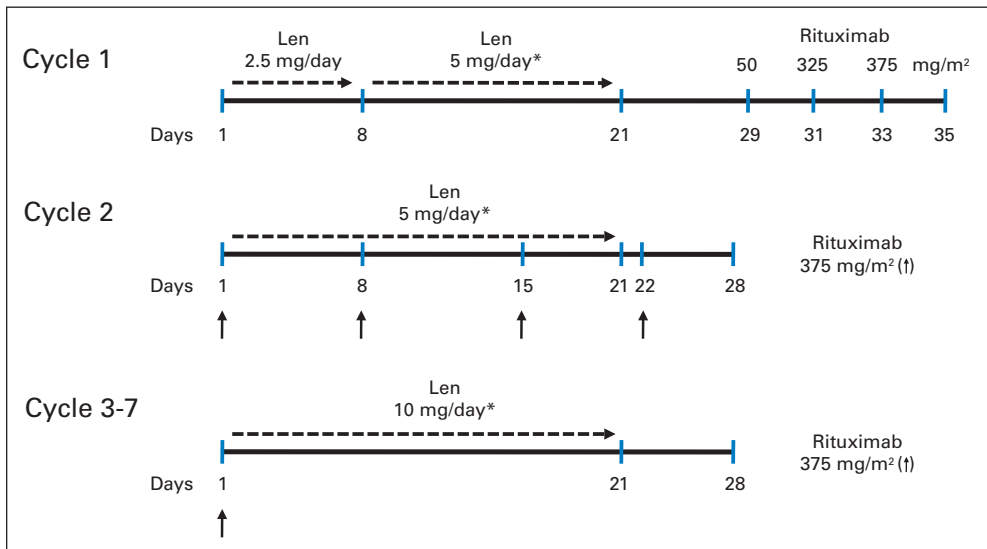
Fig A1. CRC014 treatment schema. Lenalidomide (Len) was initiated at a dose of 2.5 mg and, on day 8 of therapy, in the absence of adverse events that were G2 or higher in severity, was escalated to 5 mg. Lenalidomide was administered 21 days per cycle followed by a period of rest. Cycle 1 was 35 days and cycles 2 to 7 were 28 days. Lenalidomide could be escalated to a maximum of 10 mg, but no earlier than cycle 3. Rituximab was initiated following the 3-week lenalidomide single-agent lead in at the end of cycle 1 and continued weekly through cycle 2 and thereafter once per cycle for a total of seven cycles of the combination. (*) Len dose was increased to the level indicated as tolerated.