Original Article

Tumor Flare Reaction Associated With Lenalidomide Treatment in Patients With Chronic Lymphocytic Leukemia Predicts Clinical Response

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BACKGROUND: In patients with chronic lymphocytic leukemia (CLL), treatment with lenalidomide induces a unique, previously uncharacterized, immune response called tumor flare reaction (TFR). The clinical significance of this reaction remains unknown. **METHODS:** Forty-five patients with CLL who were treated with lenalidomide in a phase 2 clinical trial were evaluated for the clinical features, intensity, and duration of TFR. Correlation was made with tumor response and the immune cellular microenvironment. Steroids for the prophylaxis of TFR was not given to patients in Group A (n = 29) whereas patients in Group B (n = 16) received low-dose prednisone as well as a slow dose escalation of lenalidomide for the prevention of TFR. **RESULTS:** Thirty (67%) patients experienced a TFR, with a grade 2 or 3 reaction (according to National Cancer Institute Common Toxicity Criteria [version 3.0]) observed in 33% of patients (47% in Group A and 9% in Group B; $P = .05$). The median time to onset of the TFR was 6 days, and was longer in the patients receiving prophylaxis (4 days vs 9 days, respectively; $P = .01$). A complete response was observed in 7 of 30 (23%) patients with TFR and 1 of 15 (7%) patients without TFR. The median progression-free survival was 19.9 months and 19.4 months, respectively, for patients with versus those without TFR ($P = .92$). **CONCLUSIONS:** TFR is a unique immune-mediated phenomenon noted with lenalidomide treatment only in patients with CLL that correlates with clinical response. It can be effectively managed with anti-inflammatory agents. ***Cancer*** **2011;117:2127–35.**

KEYWORDS: chronic lymphocytic leukemia, lenalidomide, tumor flare reaction, prophylaxis, nonsteroidal anti-inflammatory drugs.

Lenalidomide is an immunomodulatory drug that has antitumor activity in multiple myeloma and myelodysplastic syndromes.[1,2] Although a full understanding of lenalidomide's mechanism of action still needs to be defined, preclinical evaluations have reported on its ability to modulate the immunological microenvironment via activation of the T and natural killer (NK) cells[3] as well as by down-regulation of several critical prosurvival cytokines in the tumor microenvironment, including interleukin-6, tumor necrosis factor-α, and vascular endothelial growth factor.[4] In addition, it may affect antigen-presenting cell function and antibody-dependent cell-mediated cytotoxicity. Because of its effect on the tumor microenvironment as well established antitumor activity in other B-cell cancers, we investigated its clinical efficacy in patients with chronic lymphocytic leukemia (CLL). In a phase 2 clinical trial, we observed significant clinical responses to treatment with lenalidomide alone (updated results: an overall response rate of 58% with an 18% complete response [CR] rate) in patients with recurrent or refractory CLL.[5] Although the majority of side effects noted during the conduct of the

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clinical study[6] were consistent with those reported previously for lenalidomide, tumor flare reaction (TFR) was a unique observation[6,7] that to our knowledge was not reported in studies of lenalidomide in the treatment of other disease entities.[1,2,8] It is interesting to note that in patients with CLL, this reaction was observed during treatment with both thalidomide and lenalidomide.[7,9,10]

Clinically, TFR presents as an acute inflammatory reaction that primarily involves tumor-bearing sites. Although our initial study reported on this phenomenon as a possible side effect, to our knowledge to date there has been no account that completely characterized the clinical features and the natural course of TFR. Because TFR is associated with morbidity and, more importantly, mimics disease progression, its correct diagnosis and management are critical for its effective use in patients with CLL. More importantly, the clinical significance of this immunological phenomenon and its correlation with the antitumor effects of lenalidomide remain unknown. In this article, we reported to our knowledge for the first time the clinical features of lenalidomide-associated TFR, described its effective management strategies, and demonstrated that the occurrence of TFR is an important immunological phenomenon that correlates with clinical response in patients with CLL who are treated with lenalidomide.

MATERIALS AND METHODS

Clinical Trial Design

Forty-five patients with recurrent or refractory CLL were enrolled in a phase 2 clinical trial.[6] The first 29 patients enrolled received single-agent lenalidomide at a dose of 25 mg orally for 21 days of a 28-day schedule (Group A). These individuals also received allopurinol (at a dose of 300 mg daily, 2 to 3 days before treatment and continued for up to 14 days) for the prevention of tumor lysis syndrome (TLS). In this study, TLS was observed in only 2 patients. We thereafter investigated a lower starting dose of lenalidomide with subsequent intrapatient dose escalation. Subsequently, 16 patients who were enrolled initiated treatment with lenalidomide at a dose of 10 mg/day and escalated by 5 mg every 1 to 2 weeks to a maximum of 25 mg (Group B). Furthermore, the first 29 patients enrolled did not receive TFR prophylaxis (Group A). However, because of frequent TFR, the subsequent 16 patients enrolled received low-dose oral prednisone (20 mg daily for 5 days followed by 10 mg for 5 days) as TFR prophylaxis from treatment on Days 1 to 10 of Cycle 1 (Group B). TFR prophylaxis was not given in subsequent cycles. The severity of the TFR was graded according to the National Cancer Institute Common Toxicity Criteria (version 3.0) (ie, grade 1, mild pain not interfering with function; grade 2, moderate pain [pain or analgesics interfering with function, but not interfering with activities of daily living (ADL)]; grade 3, severe pain [pain or analgesics interfering with function and ADL]; and grade 4, disabling pain). Treatment with lenalidomide can result in the development of a rash, independent of TFR. Therefore, patients in whom the only presenting feature was a rash without any other sign of TFR were excluded from the TFR group but were included in the non-TFR patient group.

Immune Effector Cell Profile

Peripheral blood was obtained at baseline for analysis of the immune cell profile. Samples were analyzed immediately at the time of harvesting for NK and T-cell repertoire using flow cytometry with the following antibody panels (all provided by BD Biosciences, San Jose, Calif): CD16/CD56/CD45 panel (NK cells), CD3/CD4/CD45 panel (CD4+ T lymphocytes), and CD3/CD8/CD45 panel (CD8+ T lymphocytes). All antibodies were titered and used at saturating concentrations.

Statistical Analysis

Comparisons between the 2 groups were performed with the following statistical analysis:the Wilcoxon-Mann-Whitney U test was used for complete continuous data, the log-rank test was used for censored time-to-event data, and the chi-square test was used for discrete variables. If the time-to-event curves crossed, the modified Kolmogorov-Smirnov test for censored data was applied.[11] The Kaplan-Meier method was used to estimate time-to-event distributions. If the assumptions of the chi-square test were violated, the Fisher exact test was applied. For correlative studies, the Wilcoxon rank sum test was used for bi-level comparison, a nonparametric analysis assessing whether 2 independent samples of marker values come from the same distribution. The tri-level comparisons were made using the Kruskal-Wallis test, testing for equality of medians across $\geq$3 groups. Any P value $<.05$ was considered to be statistically significant. All calculations were performed with SAS statistical software (version 9.1; SAS Institute Inc, Cary, NC).

Clinical Feature of TFR

The TFR often included a sudden onset (within hours of the first dose) of painful and tender enlargement of

Table 1. Clinical Features of TFR Observed in Patients Experiencing the Reaction After Lenalidomide Therapy

Feature	All Patients N = 45 (%)	All Patients With Flare N = 30 (%)	Group A[a] N = 29 (%)	Group B[b] N = 16 (%)
Lymph node swelling	28 (62)	28 (93)	18 (62)	10 (63)
Fever	5 (11)	5 (17)	3 (10)	2 (13)
Rash	10 (22)	10 (33)	6 (21)	4 (25)
Bone pain	1 (2)	1 (3)	1 (3)	0 (0)
Increase in ALC	6 (13)	6 (20)	3 (10)	3 (19)

TFR indicates tumor flare reaction; ALC, absolute lymphocyte count.
[a] Group A received single-agent lenalidomide therapy (at a dose of 25 mg orally for 21 days of a 28-day schedule) with no TFR prophylaxis.
[b] Group B received slow dose escalation of lenalidomide (at a dose of 10 mg/day and escalated by 5 mg every 1 to 2 weeks to a maximum of 25 mg) and TFR prophylaxis with low-dose prednisone (at a dose of 20 mg daily for 5 days followed by 10 mg for 5 days).

disease-involved lymph nodes, the spleen, and/or liver, which was often accompanied by low-grade fever, localized erythema, and/or generalized rash. The rash was often diffuse, erythematous, nonpruritic, and maculopapular in character. One patient also experienced bone pain concurrently with TFR that resolved with resolution of the TFR. Six of 45 patients also had an increase in their circulating leukemic cells (CD19+/CD5+) in the peripheral blood that eventually resolved with continued treatment (Table 1). The clinical characteristics of a typical TFR are illustrated in Figure 1.

RESULTS

Patients

A total of 45 patients were enrolled on this clinical study. The patients had a median age of 64 years and had received a median of 3 prior treatments. Among the patients enrolled, 80% were male, 64% had advanced Rai stage, and 51% overexpressed zeta-associated protein (ZAP)-70 protein (Table 2).

Occurrence of TFR

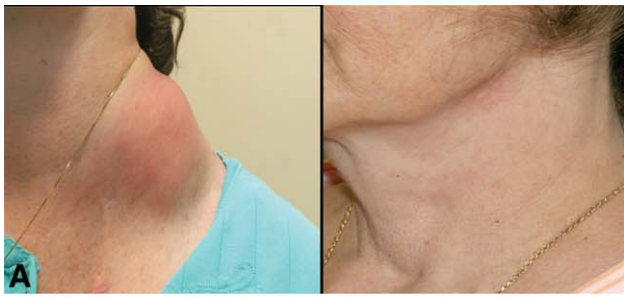
Thirty patients (67%) experienced a TFR, 20 of which (67%) were grade 1, 7 (23%) were grade 2, and 3 (10%) were grade 3 (Table 2). The onset of the TFR was recorded as early when it occurred within 24 hours of the first dose of lenalidomide and, in >90% of cases, was noted only during the first treatment cycle. The median time interval from the initiation of lenalidomide treatment to the occurrence of a TFR was 6 days (range, 0 days-56 days). The median time to resolution of the TFR was 14 days (95% confidence interval [95% CI], 10 days-26 days). In Group A (n = 29 patients), 18 (62%) patients experienced TFR at the 25-mg dose and 1 patient developed TFR at the 15-mg dose. The median lenalidomide dose at the time of TFR in Group A was 25 mg (range, 15 mg-25 mg). The majority of TFRs occurring in Group A were of grade 1 in severity (53%), followed by 37% with grade 2 and 11% with grade 3.

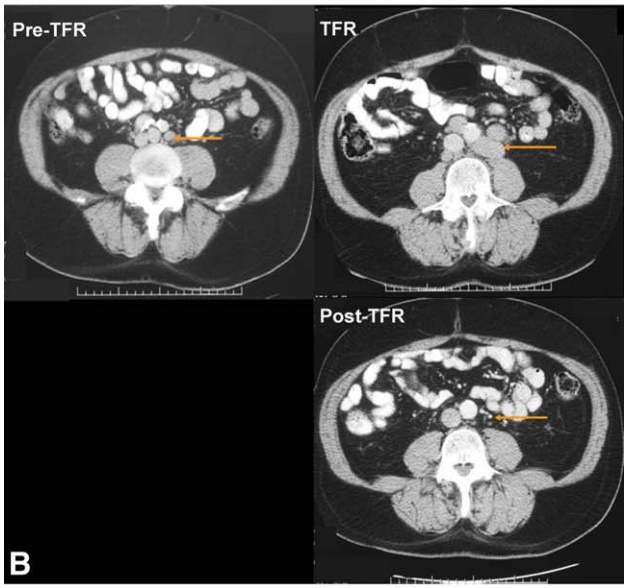
Of the patients in Group B (starting dose of 10 mg), only 12 patients reached the 25-mg dose through dose escalation. Six patients required 42 days and 3 patients required >100 days (105 days, 133 days, and 182 days, respectively) to reach the 25-mg dose. Four patients did not reach 25 mg. The time to escalate to 25 mg of lenalidomide was computed (using censored data for patients who did not reach the 25-mg dose). The median time to reach the 25-mg dose of lenalidomide for these 16 patients was 46 days from the initiation of treatment (95% CI, 42 days-63 days). Among patients starting at the 10-mg dose (n = 16), 9 experienced TFR at the 10-mg dose and 2 patients experienced a TFR at a dose of 20 mg of lenalidomide. The median lenalidomide dose at the time of TFR in Group B was 10 mg (range, 10 mg-20 mg). In Group B, nearly all TFRs (91%) were grade 1, with none reported as grade 2 and only 1 patient (9%) experiencing a grade 3 TFR.

Risk Factors for TFR

We further analyzed the baseline clinical features of patients to ascertain risk factors predictive of TFR. We observed a statistically significant difference in the median age of the patients who either developed or did not develop a TFR (61 years vs 71 years, respectively; $P = .004$ using the 2-sided Wilcoxon test). Furthermore, patients who developed a TFR tended to have a higher stage of disease (73% with Rai stage III-IV disease vs 47% incidence),

although this did not reach statistical significance ($P = .08$). To determine whether the increased incidence of TFR in patients with a higher disease stage was because of bulky disease, we correlated the presence of bulky disease (defined as any single lymph mode mass measuring 5 cm in any single dimension) with the clinical disease stage of the patients. The incidence of bulky disease in patients with Rai stage I, II/III, and IV disease was 100%, 50%, and 62%, respectively. There was no significant difference noted with regard to the incidence of TFR among patients with bulky versus nonbulky disease (65% [n = 17] vs 63% [n = 5]; $P = 1.00$). Furthermore, there was no significant association noted between TFR and gender, number of prior therapies, baseline absolute lymphocyte count, or ZAP-70 status in either group ($P > .1$).





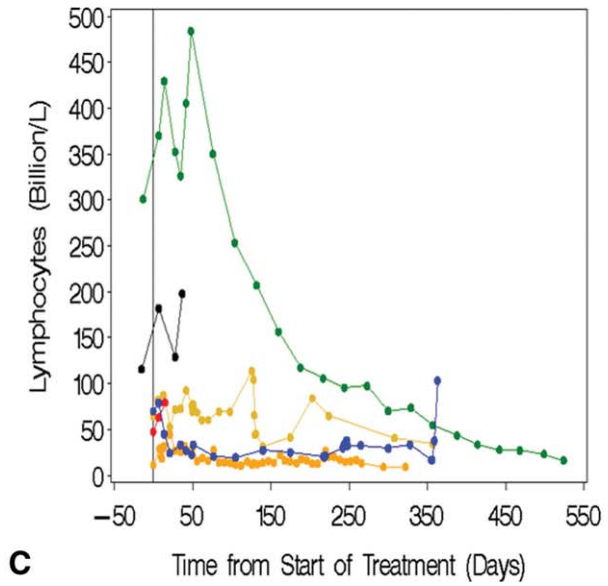


Figure 1. Clinical features of tumor flare reaction (TFR) are shown. (A) TFR manifesting as a tender increase in the size of a left cervical lymph node with overlying erythema after 7 days of therapy with lenalidomide is shown in a patient with chronic lymphocytic leukemia. The TFR resolved completely after 2 months with continued therapy. (B) TFR presenting as lower back pain because of a sudden increase in the size of retroperitoneal lymph nodes after 7 days of treatment with lenalidomide is shown. It completely resolved after continued treatment. The arrow identifies retroperitoneal lymph nodes noted on computed tomography scan at baseline (pretreatment), during TFR, and at the time of resolution. (C) In some patients, TFR can also present as an increase in the absolute lymphocyte count (ALC) in the peripheral blood. Six patients demonstrated an increase in peripheral blood ALC as a manifestation of TFR. Kinetics of ALC in the peripheral blood circulation of these patients is noted in this figure showing an initial increase in the ALC with lenalidomide treatment, which subsequently resolved with continued treatment.

Effect on Immune Cellular Microenvironment

Lenalidomide modulates the components of the immune cellular microenvironment and this might be an important mechanism underlying development of the TFR. Therefore, we investigated in a subset of patients (n = 18) whether baseline levels of immune effector cells including NK and T-lymphocyte subsets in peripheral blood correlated with the occurrence and intensity of TFR.

NK Cells

Pretreatment NK cell levels were analyzed as described earlier. We investigated whether pretreatment NK cell levels predict the intensity of a TFR. The median pretreatment NK cell level in patients with grade 1 versus those with grade 2 and 3 TFRs was 264 cells/mm^3 (range, 36-1355) and 579 cells/mm^3 (range, 164-2439), respectively (Fig. 2A). Using the Wilcoxon rank sum test, we found a statistically significant relation between pretreatment NK cell level and the intensity of the TFR ($P = .05$). We observed that individuals who developed more intense TFRs (grades 2 and 3) had a higher baseline NK cell level

Table 2. Characteristics of Patients With CLL Treated With Lenalidomide

Characteristic	All Patients N = 45	Group A[a] N = 29	Group B[b] N = 16	P[c]
Median age (range), y	64 (42-75)	63 (47-74)	65 (42-75)	.79
Sex, no. (%)				.46
Male	36 (80)	22 (76)	14 (88)	
Female	9 (20)	7 (24)	2 (12)	
Rai stage, no. (%)				.84
0-II	16 (36)	10 (34)	6 (38)	
III-IV	29 (64)	19 (66)	10 (62)	
Median no. of prior treatments (range)	3 (1-9)	3 (1-9)	1.5 (1-6)	.03
Median LDH, IU/L (range)	565 (106-1450)	561 (106-1429)	601 (134-1450)	0.5
Median baseline ALC, $\times 10^9$/L (range)	43.6 (0.7-339)	45.9 (0.7-339)	35.1 (3.5-327)	.57
ZAP-70, no. (%)				1.00
Positive	23 (51)	15 (52)	8 (50)	
Negative	13 (29)	8 (28)	5 (31)	
NA	9 (20)	6 (21)	3 (19)	
Allopurinol prophylaxis, no. (%)	35 (78)	22 (76)	13 (81)	1.00
Administered	1 (2)	1 (3)	0	
Not given/unknown	9 (20)	6 (21)	3 (19)	
TFR, no. (%)	30 (67)	19 (66)	11 (69)	.83
Grade 1	20 (67)	10 (53)	10 (91)	.05
Grade 2	7 (23)	7 (37)	0 (0)	
Grade 3	3 (10)	2 (11)	1 (9)	
Median time to onset (range), d	6 (0-56)	4 (0-38)	9 (2-56)	.01
Median duration (95% CI), d	14 (10-26)	13 (10-25)	28 (14-97)	.16
Clinical response				.36
ORR, %	53	48	63	
CR, no. (%)	8 (18)	5 (17)	3 (19)	
Treatment discontinuation[d]	None	None	None	—

LDH indicates lactate dehydrogenase; ALC, absolute lymphocyte count; ZAP-70, zeta-associated protein-70; NA, not available; TFR, tumor flare reaction; 95% CI, 95% confidence interval; ORR, overall response rate; CR, complete response.

[a] Group A received single-agent lenalidomide therapy (at a dose of 25 mg orally for 21 days of a 28-day schedule) with no TFR prophylaxis.

[b] Group B received slow dose escalation of lenalidomide (at a dose of 10 mg/day and escalated by 5 mg every 1 to 2 weeks to a maximum of 25 mg) and TFR prophylaxis with low-dose prednisone (at a dose of 20 mg daily for 5 days followed by 10 mg for 5 days).

[c] Comparison between Groups A and B.

[d] None of the patients required discontinuation of therapy for TFR.

compared with those with less intense or no TFRs (grades 0 and 1). Furthermore, individuals with either normal or elevated baseline NK cell levels appeared to have a better quality of response to lenalidomide; among the 5 patients who achieved a CR, all but 1 patient had a normal or elevated baseline NK cell level. The median pretreatment NK cell level among patients with a clinical response (CR and partial response) compared with those with no response [all others]) was 448 cells/mm^3 (range, 100-2439) and 303 cells/mm^3 (range, 36-609), respectively. Clinical outcome (response vs no response) was compared using a logistic regression model and did not conclusively establish an association between pretreatment NK cell level and clinical outcome ($P = .24$). The 2 patients with stable disease also had elevated baseline NK cell levels, indicating that other as yet unidentified factors may also be important in the antileukemic effect of lenalidomide in patients with CLL.

T Cells

Pretreatment T suppressor (CD4+) and T cytotoxic (CD8+) cells were analyzed as described earlier (Figs. 2B-2D). A logistic regression model was used to determine an effect from T cells with respect to TFR grade (grade 1 vs grade 2 and grade 3). The logarithmic transform of the T cell count was used. The median number of CD4+ cells for the grade 1 TFR group (n = 12) was 729 cells/mm^3 (range, 11-2073) versus 598 cells/mm^3 (range, 182-5689) for the grade 2 (n = 6) and grade 3 TFR groups, respectively ($P = .34$). The median number of pretreatment CD8+ cells in patients with grade 1 versus those with

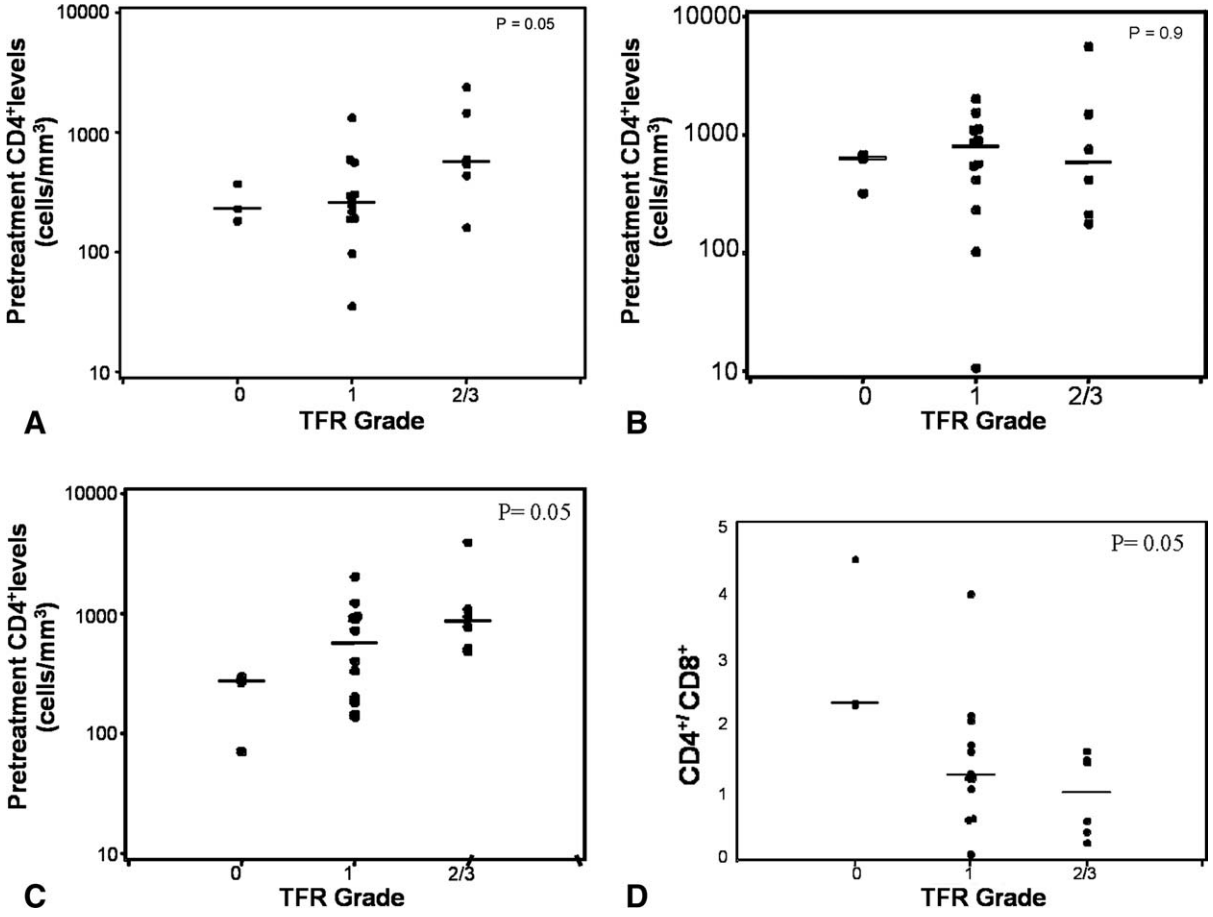


Figure 2. The effect of immunologic cells on the development of tumor flare reaction (TFR) was investigated. Baseline immune effector cell levels were found to be correlated with the intensity of the TFR using a logistic regression model. (A) Pretreatment natural killer (NK) (CD56+/CD16+) cell level is shown. (B) Pretreatment T-helper (CD4+/CD45+) cells are shown. (C) Pretreatment T-cytotoxic (CD8+/CD45+) cells were analyzed using flow cytometry. The median number of cells in each group is noted in each panel on the plot with a short horizontal line.

grade 2 and grade 3 TFRs was 575 cells/mm^3 (range, 141-2080) and 873 (range, 494-4021), respectively ($P = .15$). Using the Kruskal-Wallis test, we observed no significant relation between of baseline CD4+ counts and the intensity of the TFR ($P = .9$). It is interesting to note that the baseline level of CD8+ cytotoxic cells was found to be correlated with the intensity of the TFR ($P = .05$). In addition, the CD4/CD8 ratio at baseline was found to be a strong indicator of the intensity of the TFR ($P = .01$).

Management of TFR

In our clinical trial, management of the TFR included the use of a nonsteroidal anti-inflammatory drug (NSAID) (ibuprofen at a dose of 400-600 mg every 4-6 hours) with or without oral morphine sulfate (5-10 mg every 4-6 hours as needed) for additional pain control. Overall, 11 (37%) of the 30 patients exhibiting a TFR required treatment with ibuprofen; 7 (23%) were in Group A and 4 (13.3%) were in Group B. Only 3 (10%) patients (2 in Group A and 1 in Group B) received supplemental morphine sulfate for pain control. No patients required discontinuation of treatment or a dose reduction of lenalidomide for a TFR. The high incidence of TFR in Group A prompted us to investigate the prevention of this reaction with the use of low-dose corticosteroid therapy. None of the patients in Group A was given steroids either for the prevention or treatment of a TFR.

Correlation Between TFR and Response to Therapy

Lenalidomide induced a rapid and sustained decline in the peripheral blood tumor burden.[6] Clinical response

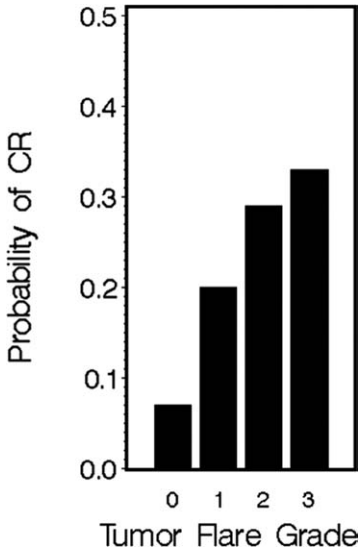


Figure 3. The intensity of tumor flare reaction with lenalidomide in patients with chronic lymphocytic leukemia and the probability of achieving a complete response (CR) are shown.

was compared among patients with or without a TFR. Despite a low sample size in our analysis, the probability of achieving a CR with lenalidomide alone was found to be correlated with the intensity of the TFR (Fig. 3). At the time of last follow-up, 8 patients had achieved a CR (5 in Group A and 3 in Group B). Among the 15 patients without a TFR, only 1 CR (7%) was observed versus 7 (23%) patients in the TFR group. Among patients with a grade 1 TFR, there were 4 CRs in 20 patients (20%); among patients with a grade 2 TFR, there were 2 CRs in 7 patients (29%); and among those with a grade 3 TFR, there was 1 CR in 3 patients (33%). The median progression-free survival (PFS) for patients with or without a TFR was 19.9 months and 19.4 months, respectively ($P = .92$).

Impact of Steroid Prophylaxis and Slow Dose Escalation

The impact of slow dose escalation of lenalidomide and low-dose corticosteroid prophylaxis was evaluated by comparing Groups A and B. Nineteen (66%) patients in Group A and 11 (69%) patients in Group B developed a TFR ($P = .83$, chi-square test). Among these individuals, grade 2 and 3 TFRs were observed in 9 patients (47%) and 1 patient (9%) n Groups A and B, respectively ($P = .05$). The median time to the onset of the TFR was 4 days in Group A and 9 days in Group B ($P = .01$). The median time to resolution of a TFR was 13 days in Group A and 26 days in Group B ($P = .16$). We further evaluated the impact of steroids on PFS in patients treated with lenalidomide. The median PFS for patients who did not receive corticosteroids prophylaxis (Group A) compared with those who did (Group B) was 23 months versus 17.8 months, respectively ($P = .74$ by the log-rank test and $P = .88$ using the modified Kolmogorov-Smirnov test). Our analysis failed to demonstrate a statistically significant difference in PFS.

DISCUSSION

Lenalidomide is clinically active as a single agent in patients with CLL. Two clinical studies in patients with recurrent or refractory CLL reported response rates of 58% and 32%, respectively, with lenalidomide monotherapy.[5,12] In the current study, TFR, an adverse event that to our knowledge has not been reported with lenalidomide treatment in patients with other disease entities, occurred in 67% of individuals with CLL who were treated with lenalidomide. These rates are lower than observed in the previous study by Andritsos et al, who reported serious TFRs in 3 of 4 patients (75%), although the differences in lenalidomide dose, TFR prophylaxis administration, study population, and total patient numbers could account for these differences.[13]

In the current study, the use of an NSAID, ibuprofen, was effective in managing the pain associated with TFR and rarely oral morphine sulfate was administered for severe pain. We observed that prophylaxis with prednisone decreased the severity but not the incidence of TFR. In our experience, TFR was well tolerated, with none of the patients requiring discontinuation or a dose reduction of therapy.

Lenalidomide-associated TFR was characterized by a proinflammatory clinical picture suggestive of an immune-mediated tumor recognition phenomenon. We note that the immunomodulatory activity of lenalidomide in vivo may be integrally related to the development of the TFR.[14] The presence of an adequate amount of NK cells in peripheral blood circulation appeared to be correlated with the intensity of the TFR and also improved the response to therapy. Pre-existing immune factors (such as NK cells) that are associated with clinical response to lenalidomide suggest that lenalidomide requires immune components for its antitumor activity. We observed a higher intensity of TFR in patients who initiated therapy with lenalidomide at a higher dose and without prophylaxis and the intensity of the TFR appeared to be correlated with a higher probability of achieving a CR. The

exact clinical impact of TFR remains uncertain; although our analysis was limited by small sample size, it did not demonstrate any benefit in the PFS despite a higher CR rate in the TFR group. A potential clinical significance of TFR maybe a high probability of achieving a CR, which in turn may be associated with an improved PFS. In the clinical trial reported by Ferrajoli et al, no association between TFR and response was noted.[12] The disparity in this observation may reflect different patient populations and variable dosing schedules as well as starting dose. Ongoing clinical trials with a large sample size should be able answer this question.

The identification and careful characterization of a TFR is important in the treatment of CLL patients with lenalidomide to avoid unnecessary morbidity or the premature discontinuation of effective therapy. We recommend close monitoring of patients with CLL for signs of TFR (ie, enlargement of lymph nodes, rash, and changes in the ALC) during the first 10 days of lenalidomide therapy. It is particularly important to monitor lymph nodes close to the liver and kidneys. If a TFR occurs, we recommend treatment with an NSAID such as ibuprofen (at a dose of 400- 600 mg every 6 hours), with steroid use considered only in cases of more intense TFRs. Although in the current study we did not need to withhold or reduce the dose of lenalidomide out of concerns for TFR, in severe cases of TFR this may be warranted along with steroid treatment. Alternatively, a slow dose escalation strategy such as that used in Group B in the current study may reduce the intensity of TFR and should be considered. The dose of lenalidomide can be increased again once the TFR subsides. In severe cases, therapy with low-dose corticosteroids can be used to decrease the severity of the TFR although, as previously discussed, this can potentially undermine antitumor responses. In our experience, it is not necessary to discontinue lenalidomide therapy for a TFR.

In conclusion, the occurrence of a TFR appears to result from the immune-stimulating activity of lenalidomide in patients with CLL. Preclinical evaluations suggest that 1 possibly important effect of lenalidomide may be the activation of T cell-mediated anti-CLL effects. Although these in vitro observations add new information regarding the potential immunologic effect of lenalidomide and thus may explain clinical immunologic phenomenon such as TFR, clinical validations are pending.[15] Our clinical observation that the intensity of the TFR is correlated with the probability of achieving a CR is interesting and requires further validation and may suggest that this is an important phenomenon linked to lenalidomide's response in patients with CLL. Because of its high incidence, its considerable morbidity, and its clinical presentation resembling progressive disease, the early recognition and accurate diagnosis of TFR is imperative for the effective use of lenalidomide in patients with CLL. Further studies are warranted to elucidate the underlying pathophysiological mechanisms responsible for the development of the lenalidomide-associated TFR in patients with CLL.

CONFLICT OF INTEREST DISCLOSURES

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REFERENCES

1. Richardson PG, Schlossman RL, Weller E, et al. Immunomodulatory drug CC-5013 overcomes drug resistance and is well tolerated in patients with relapsed multiple myeloma. *Blood*. 2002;100:3063-3067.
2. List A, Kurtin S, Roe DJ, et al. Efficacy of lenalidomide in myelodysplastic syndromes. *N Engl J Med*. 2005;352:549-557.
3. Chang DH, Liu N, Klimek V, et al. Enhancement of ligand-dependent activation of human natural killer T cells by lenalidomide: therapeutic implications. *Blood*. 2006;108:618-621.
4. Mitsiades CS, Mitsiades N. CC-5013 (Celgene). *Curr Opin Investig Drugs*. 2004;5:635-647.
5. Chanan-Khan MK, Whitworth A, Lawrence D, et al. Clinical activity of lenalidomide in relapsed or refractory chronic lymphocytic leukemia (CLL) patients: updated results of a phase II clinical trial. *Leuk Lymphoma*. 2007;48(suppl 1):S166.
6. Chanan-Khan A, Miller KC, Musial L, et al. Clinical efficacy of lenalidomide in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase II study. *J Clin Oncol*. 2006;24:5343-5349.
7. DiMiceli L, Miller K, Rickert M, et al. Characterization of IMiDs (immunomodulating agents) induced "flare reaction" in patients with chronic lymphocytic leukemia (CLL) and correlation with changes in serum cytokine levels [abstract]. *Blood*. 2005;106:5049.
8. Rajkumar S, Jacobus S, Callander N, et al. Phase III trial of lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone in newly diagnosed multiple myeloma (E4A03): a trial coordinated by the Eastern Cooperative Oncology Group [abstract]. *J Clin Oncol*. 2007;25:LBA8025.
9. Chanan-Khan A, Miller KC, Takeshita K, et al. Results of a phase 1 clinical trial of thalidomide in combination with

fludarabine as initial therapy for patients with treatment-requiring chronic lymphocytic leukemia (CLL). *Blood*. 2005;106:3348-3352.
10. Kay N, Geyer S, Yaqoob I, Phyliky R, Kutteh L, Li C-Y. N9986: a phase II trial of thalidomide in patients with relapsed chronic lymphocytic leukemia. *Leuk Lymphoma*. 2009;50:588-592.
11. Fleming T, O'Fallon J, O'Brien P, Harrington D. Modified Kolmogorov-Smirnov test procedures with application to arbitrarily right-censored data. *Biometrics*. 1980;36:607-625.
12. Ferrajoli A, Keating M, Wierda W, et al. Lenalidomide is active in patients with relapsed/refractory chronic lymphocytic leukemia carrying unfavorable chromosomal abnormalities [abstract]. *Blood*. 2007;110:11.
13. Andritsos LA, Johnson AJ, Lozanski G, et al. Higher doses of lenalidomide are associated with unacceptable toxicity including life-threatening tumor flare in patients with chronic lymphocytic leukemia. *J Clin Oncol*. 2008;26:2519-2525.
14. Padmanabhan S, Ersing N, Wallace P, et al. First clinical evidence of in vivo Natural Killer (NK) cell modulation in chronic lymphocytic leukemia (CLL) patients treated with lenalidomide [abstract]. *Blood*. 2006;108:2109.
15. Ramsay AG, Johnson AJ, Lee AM, et al. Chronic lymphocytic leukemia T cells show impaired immunological synapse formation that can be reversed with an immunomodulating drug. *J Clin Invest*. 2008;118:2427-2437.