Low-dose radiotherapy in diffuse large B-cell lymphoma

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Abstract

Low-dose radiotherapy (LDRT) given in 2 × 2 Gy is a highly effective and safe treatment for palliation of indolent lymphomas. Otherwise, very little regarding the use of LDRT for diffuse large B-cell lymphoma (DLBCL) has been investigated. We designed a phase 2 trial of LDRT in patients with DLBCL with indication for palliative radiation. Low-dose radiotherapy was administered on symptomatic areas only. Clinical response was assessed 21 days after LDRT and defined as reduction >50% of maximum diameter of the radiated lesions. Quality of life was scored by the European Organisation for Research and Treatment of Cancer QLQ-C30 questionnaire. Tumor subtype (germinal center B-cell type versus activated B-cell type) and the presence of *TP53* mutations in pathologic specimens of the target lesion were also evaluated. Twenty-three of twenty-five radiated patients were evaluable for response, and 2 died of disease before the visit at 21 days. The overall response rate was 70% (16 of 23 patients), with 7 complete responses and 9 partial responses (mean duration of response, 6 months; range, 1-39 months). Fifteen patients answered to the QLQ-C30 questionnaires, and an improved quality of life was documented in 9 cases. *TP53* mutations were detected in 2 of 6 (33%) nonresponders and in none of the responders (P = .12). Germinal center B-cell type responded better than activated B-cell type (response rate was 83% and 29%, respectively, P = .01). These findings indicate that LDRT is effective for palliation in patients with DLBCL.

KEYWORDS

DLBCL, low-dose radiotherapy, palliation, quality of life, *TP53*

1 | INTRODUCTION

Hematologic malignancies are radio-sensitive, and usually, a radiation dose of 24 to 40 Gy is recommended for local control (LC). Starting from the 1990s, low-dose radiotherapy (LDRT) has been gradually introduced for palliation of lymphomas, showing that among non-Hodgkin lymphomas (NHLs), low-grade subtypes can be controlled by very low doses of radiotherapy, in the order of 4 Gy.[1]

Although literature data support the efficacy of LDRT for indolent NHLs, with an overall response rate greater than 80%, without significant toxicity,[2–9] only 3 studies have experimented LDRT in aggressive lymphomas showing that LDRT may also be effective in these patients,[3,5,6] despite the limited number of cases included in the analyses,[5] and the inclusion of mantle cell lymphomas among the aggressive forms.[3,6] In this context, very little regarding the use of LDRT in diffuse large B-cell lymphoma (DLBCL) has been investigated. In an attempt to obviate these limited experiences, we conducted a phase 2 trial to assess the efficacy of LDRT in DLBCL. We report here the final results of this phase 2 trial of which an interim analysis was presented at 2014 ASTRO meeting.[10]

2 | METHODS AND MATERIALS

2.1 | Study population

Our trial was a single-institution, single-arm prospective phase 2 clinical trial focused on patients with DLBCL. Patients with histologically proven diagnosis of DLBCL with indication for palliative radiotherapy were approached for this trial. No limits of age or performance status were applied. Patients with concurrent chemotherapy or chemoresistant disease were included to fit the trial with the clinical practice.

The study was approved by the local independent ethics committee and was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice. A written specific informed consent was obtained from all enrolled patients in accordance with the Declaration of Helsinki.

2.2 | Treatment regimen

Low-dose radiotherapy was delivered to a dose of 2 × 2 Gy irradiation (days 1 and 3). The planning target volume consisted of the symptomatic lesion (gross target volume) plus a 1.0-cm margin to obtain the clinical target volume plus an additional 1.0-cm setup margin.

Before treatment, the patients underwent detailed physical examination to assess the extent of disease. Comorbidity was measured using the Adult Comorbidity Evaluation-27 score.[11] The first follow-up visit was performed at 21 days after completion of LDRT to assess treatment response and toxicity, which was scored according to the Common Terminology Criteria for Adverse Events v3.0. Response evaluation was performed with cross-sectional imaging, or clinical exam for palpable lesion. Response assessment was based on the standard definitions of the World Health Organization[12] using complete response (CR), partial response (PR), stable disease, and progressive disease as response assessment criteria. Treatment efficacy was defined by a reduction greater than 50% in the maximum diameter of the radiated mass (PR + CR). To minimize the potential for undertreatment, the protocol specified that nonresponders had to be addressed to additional radiotherapy with conventional dose (20 Gy in 5 fractions or 30 Gy in 15 fractions) after the first follow-up visit.

2.3 | Quality of life evaluation

This was a quality of life (QoL)–oriented study. Quality of life was evaluated using the European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30 3.0 version questionnaire.[13] The QLQ-C30 questionnaire was self-administered to the patients at baseline and at 21 days after treatment. According to this questionnaire, a higher score in global health status and/or functional scales means a better level of functioning and/or global health status. A higher score in any of the symptom scales means a higher level of symptoms. A ≥10-point difference was considered of clinical significance.[14] The authors obtained official permission to use the QLQ-C30 questionnaire from the EORTC Data Center, Quality of Life Unit.

2.4 | Detection of biomarkers for response

Enrolled patients were asked to make available a pathologic specimen of the target lesion before LDRT to collect samples for diagnosis and biomolecular analyses. The target tissue specimen was sampled by bioptic procedures or, in selected cases, by a fine-needle aspiration cytology, whenever possible without morbidity for the patient. In the cases of deep nodal mass, only archival material of the target lesion was sampled.

DNA was extracted from archived formalin-fixed paraffin-embedded or cytological samples following standard methods (Qiagen, Hilden, Germany). Analysis of *TP53* mutations was performed as previously reported.[15] In selected cases, analysis was performed by next generation sequencing (NGS) using a MiSeq sequencer (Illumina, San Diego, California). To evaluate *TP53* mutational load, we amplified genomic DNA for *TP53* gene by multiplex polymerase chain reaction using modified primers according to the Illumina protocol, by using a high-fidelity Taq polymerase (Phusion High-Fidelity DNA Polymerase, Thermo Scientific, Waltham, Massachusetts). The obtained polymerase chain reaction products were subjected to NGS on MiSeq sequencer (Illumina, San Diego, California) to obtain approximately 1000 coverage-fold for amplicons. Results were expressed as percentage of mutated DNA. *TP53* mutations were reported when above a threshold of 5% of total sequences. Predicted structural and functional characteristics of the mutations were obtained from IARC P53 Mutation Database (http://p53.iarc.fr/).

Archival paraffin-embedded material from initial diagnostic specimens was also sampled for morphological and immunohistological investigation, according to current protocols. Sections at 4 μm were stained with hematoxylin and eosin. Sections at 2.5 μm were cut, and immunohistochemical analysis was performed in an automated system (Benchmark-XT, Ventana, Tucson, Arizona). Color was developed with 3.3′-diaminobenzidine, and slides were counterstained with the Meyer hematoxylin. Immunohistological staining for identification of cell of origin, germinal center B-cell (GCB) subtype versus activated B-cell (ABC) subtype, was performed.[16]

2.5 | Statistics

The primary end point of this phase 2 study was to assess the response rate (CR + PR) to LDRT in DLBCL patients. The number of patients required was calculated by the 2-stage Simon method.[17] The number of patients required for the first step of the phase 2 study was 14 patients, estimated by predicting a minimum response of 50% and a desired response of 75%. Both targets were chosen according to previous experiences on palliative radiation on aggressive lymphomas.[6,18] Accordingly, with less than 7 responses during the first step, the study should be closed because of no efficacy; otherwise, accrual was to continue till 23 patients were enrolled.

As a secondary end point, the association between response and tumor subtype or *TP53* functional status was evaluated. Statistical comparisons of baseline variables with response were analyzed by the χ^2 test, or the Fisher exact test. Results were considered significant for P values <.05 (2-tailed test). Time to local progression (LC), progression-free survival (PFS), and the overall survival (OS) rate were also

analyzed. Event-free rates were calculated from the end of LDRT by the Kaplan-Meyer method. For OS estimation, patients were censored for deaths by any cause; for PFS censoring, events were death by any cause or progression or the need of additional radiotherapy at the target lesion for inadequate LC.

3 | RESULTS

3.1 | Response analysis

Between August 2011 and December 2015, we assigned 25 patients with DLBCL to LDRT. The median follow-up was 5 months (range, 0-39 months). Patient characteristics are detailed in Table 1. Identification of cell of origin from initial diagnostic specimens was possible in 19 of 25 cases. The tumor subtypes were the following: 12 of 25 GCB subtype and 7 of 25 ABC subtype. The majority of patients, ie, 21 of 25 (84%), had chemoresistant disease and were deemed ineligible for chemotherapy at the time of LDRT. The median number of prior systemic therapies was 3 (0-4). Indications for palliative treatment were pain in 11 of 25 patients, functional impairment in 7 of 25 patients, and obstructive symptoms in 7 of 25 patients.

Among the 25 DLBCL patients receiving LDRT, 2 of 25 died of the disease before response assessment and were excluded from the response analysis.

The response rate to LDRT was 70%, with 7 of 23 (31%) CR and 9 of 23 (39%) PR (Table 1). Representative photographs of responding patients are shown in Figures 1, 2, and 3. Among the 16 of 23

TABLE 1 Patient characteristics at the time of LDRT (N = 25, all patients) and factors associated with response (N = 23, patients evaluable)

Characteristic	n (%)	Response to LDRT (CR + PR)	No Response to LDRT (NC + PD)	*P*
Mean age (range)	73 years (40-90)	…	…	
Overall response	…	16 (70%)	7 (30%)	
Sex				.37
Female	12 (48%)	7 (58%)	5 (42%)	
Male	13 (52%)	9 (82%)	2 (18%)	
ECOG performance status				1
0-1	8 (32%)	6 (75%)	2 (25%)	
2-3	17 (68%)	10 (67%)	5 (33%)	
Comorbidity index by ACE-27 score				.17
0-2	15 (60%)	8 (57%)	6 (43%)	
3	10 (40%)	8 (89%)	1 (11%)	
Tumor stage				1
I-II	3 (12%)	2 (67%)	1 (33%)	
III-IV	22 (88%)	14 (70%)	6 (30%)	
Chemoresistance[a]				1
Yes	21 (84%)	13 (68%)	6 (32%)	
No	4 (16%)	3 (75%)	1 (25%)	
Bulky disease[b]				.01
Yes	10 (40%)	4 (40%)	6 (60%)	
No	15 (60%)	12 (92%)	1 (8%)	
Radiation site				1
Skin	6 (36%)	4 (67%)	2 (33%)	
Nonskin	19 (64%)	12 (71%)	5 (29%)	
TP53 status				.12
Wild type	11 (44%)	8 (73%)	3 (27%)	
Mutated	2 (8%)	0	2 (100%)	
Not available	12 (48%)	7 (87%)	1 (13%)	
DLBCL subtype				.04
GCB-DLBCL[c]	12 (48%)	10 (83%)	2 (17%)	
ABC-DLBCL[d]	7 (28%)	2 (29%)	5 (71%)	
Not available	6 (24%)	1 (33%)	2 (67%)	

Abbreviations: ACE-27, Adult Comorbidity Evaluation-27; CR, complete response; DLBCL, diffuse large B-cell lymphoma; LDRT, low-dose radiotherapy; PD, progressive disease; PR, partial response; NC, no change; ECOG, Eastern Cooperative Oncology Group.

[a]Chemoresistance was defined as the failure of chemotherapy to achieve a complete or partial response, or as disease relapse after a complete response.

[b]Bulky disease was defined as >5 cm mass.

[c]Germinal center B-cell subtype.

[d]Activated B-cell subtype.

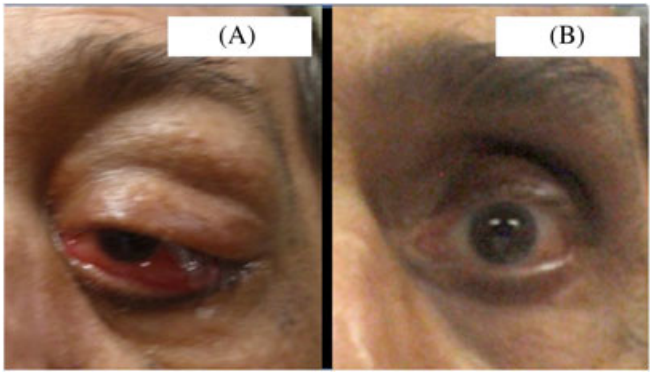

FIGURE 1 LDRT induces complete response in a 69-year-old man with orbital DLBCL. A, The patient was affected by eye irritation related to an extranodal recurrence of DLBCL after autologous self cell transplant. B, Complete response after LDRT. A significant amelioration of his global health status and social functioning was also reported on the QLQ-C30 questionnaires. DLBCL indicates diffuse large B-cell lymphoma; LDRT, low-dose radiotherapy

responder patients, the median duration of response was 6 months (range, 1-39 months), and only 2 of 16 responder patients progressed within the radiated field at the time of last follow-up visit. Two patients had mild nausea, and no other toxicities were registered. Among non-responders, 5 of 23 (22%) patients had stable disease and 2 of 23 (9%) patients had progressive disease. Of these 7 nonresponders, 4 patients went on to receive additional radiotherapy with conventional dose, 1 patient received systemic treatment for disease progression at a non-target lesion, and 2 patients were managed with best supportive care alone (Figure 4). The 1-year LC, PFS, and OS for the entire cohort were 34%, 21%, and 45%, respectively.

3.2 | Quality of life

Quality of life was assessed before treatment and 21 days after treatment. In this context, 15 patients responded to the EORTC QLQ-C30 questionnaires. Most patients had an amelioration of their QoL after LDRT (QoL outcomes of the study are reported in Table 2 and 3). The global health status rating significantly ameliorated in 9 of 15 (60%) patients, basing on the clinical criterion of considering a change of 10 or more points significant. When the functional scales (physical function, role function, emotional function, cognitive function, and social function) were evaluated, the majority of patients reported a

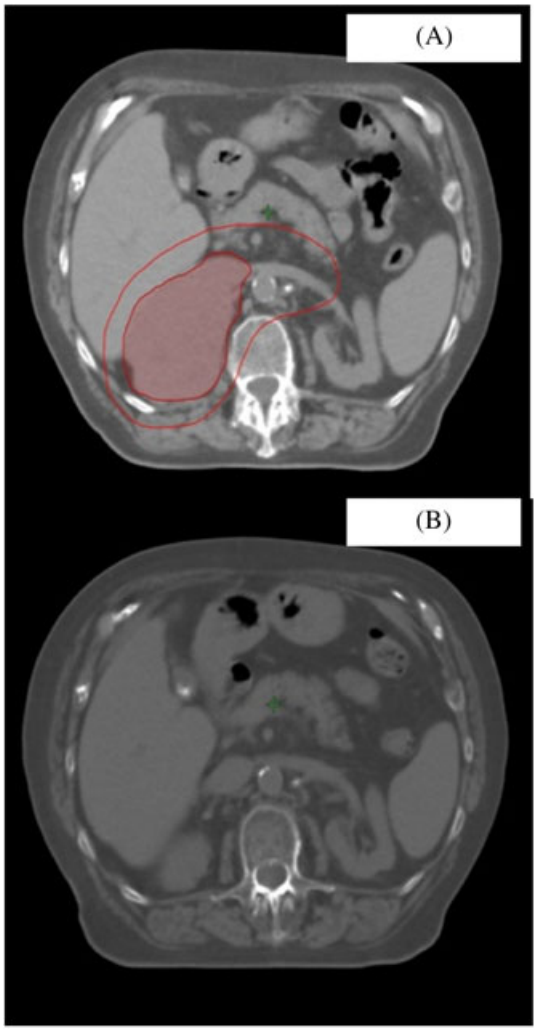

FIGURE 3 Complete response to LDRT in a case of chemoresistant DLBCL with bulky disease. A, Female patient, 79 year-old, affected by a retroperitoneal recurrence of DLBCL that enveloped the right kidney; the patient was unfit for chemotherapy due to pneumonia. The GTV and the PTV are outlined in red. B, Complete response after LDRT with reduction of pain, fatigue, appetite loss, and nausea on the QLQ-C30 symptoms scales. DLBCL indicates diffuse large B-cell lymphoma; GTV, gross tumor volume; LDRT, low-dose radiotherapy; PTV, planning target volume

significant amelioration of their role and social functioning after LDRT. Moreover, when the mono-item scales describing relevant cancer-oriented symptoms (fatigue, nausea, pain, dyspnea, insomnia, appetite,

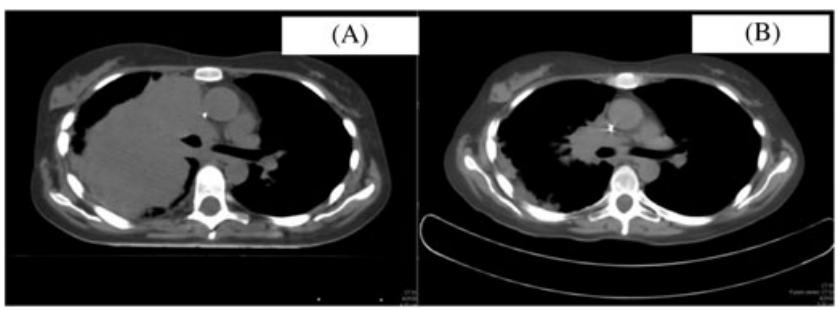

FIGURE 2 The case of a 41-year-old woman with severe dyspnea due to massive right lung involvement of DLBCL. The patient was not suitable for any chemotherapeutic regimen due to pancytopenia, and she did not accept blood transfusions. A, Whole lung LDRT was administered. B, Three weeks after the end of LDRT, the mass has reduced, with remarkable improvement in fatigue, physical functioning, and global health status, based upon the QLQ-C30 scores. DLBCL indicates diffuse large B-cell lymphoma; LDRT, low-dose radiotherapy

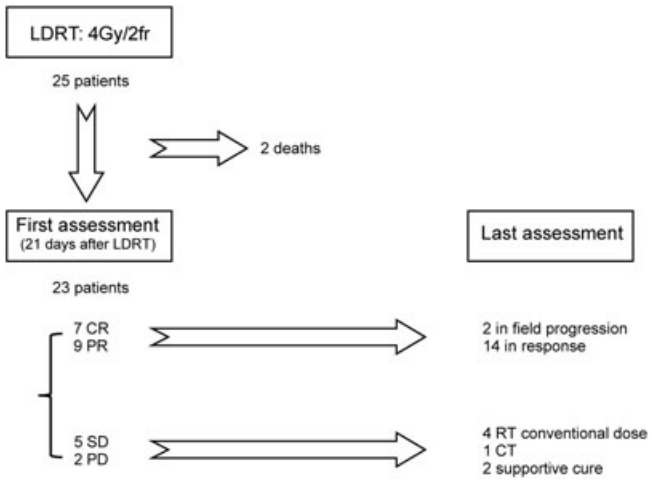

FIGURE 4 Posttreatment outcomes. CR indicates complete response; LDRT, low-dose radiotherapy; PD, progressive disease; PR, partial response; RT, radiotherapy; SD, stable disease; CT, chemotherapy

TABLE 2 The course of global health status and functional scales values featured on the EORTC QLQ-C30 questionnaire[a,b]

	No. of Patients
Global health status	
Ameliorated	9 (60%)
No change	6 (40%)
Worsened	0
Functional scales	
Physical functioning	
Ameliorated	5 (34%)
No change	8 (53%)
Worsened	2 (13%)
Role functioning	
Ameliorated	9 (60%)
No change	5 (34%)
Worsened	1 (6%)
Emotional functioning	
Ameliorated	2 (14%)
No change	12 (86%)
Worsened	0
Cognitive functioning	
Ameliorated	2 (13%)
No change	7 (47%)
Worsened	6 (40%)
Social functioning	
Ameliorated	8 (53%)
No change	4 (27%)
Worsened	3 (20%)

Abbreviation: EORTC, European Organisation for Research and Treatment of Cancer.

[a]A difference ≥ 10 points was considered of clinical significance.

[b]Percentages are calculated on the 15 patients who answered to the QLQ-C30 questionnaires.

constipation, diarrhea, and financial difficulties) were evaluated, the 53% of patients reported a significant amelioration of fatigue and pain after LDRT.

TABLE 3 The course of symptoms scales values featured on the EORTC QLQ-C30 questionnaire[a,b]

Symptoms Scales	No. of Patients
Fatigue	
Decreased	8 (53%)
No change	3 (20%)
Increased	4 (27%)
Nausea and vomiting	
Decreased	3 (20%)
No change	11 (73%)
Increased	1 (7%)
Pain	
Decreased	8 (53%)
No change	4 (27%)
Increased	3 (20%)
Dyspnea	
Ameliorated	4 (27%)
No change	8 (53%)
Worsened	3 (20%)
Insomnia	
Decreased	4 (27%)
No change	8 (53%)
Increased	3 (20%)
Appetite loss	
Decreased	6 (40%)
No change	7 (47%)
Increased	2 (13%)
Constipation	
Decreased	5 (34%)
No change	5 (33%)
Increased	5 (33%)
Diarrhea	
Decreased	2 (14%)
No change	12 (80%)
Increased	1 (6%)
Financial difficulties	
Decreased	1 (6%)
No change	12 (80%)
Increased	2 (14%)

Abbreviation: EORTC, European Organisation for Research and Treatment of Cancer.

[a]A difference ≥ 10 points was considered of clinical significance.

[b]Percentages are calculated on the 15 patients who answered to the QLQ-C30 questionnaires.

3.3 | Detection of biomarkers for response

Table 1 shows the correlations among patient characteristics and disease factors. In particular, there was a significant association between the tumor subtype and the response to LDRT (83% of patients with GCB responded to LDRT versus 29% of responders in the ABC group; $P = .04$). Moreover, bulky disease resulted as the only additional factor that impacted response in a significant way in this setting ($P = .01$).

The target lesion tissue was suitable for *TP53* genotype analyses in 14 of 23 (61%) patients (in the remaining 9 cases, no material was

available). In this subset, *TP53* functional status (wild type versus mutated) showed a borderline association with clinical response (P = .12). Analysis of predictive markers for LC demonstrated that tumor subtype and *TP53* genotype were the only 2 factors associated with LC after LDRT (1-year LC, 47% in GCB-DLBCL patients versus 0% for ABC-DLBCL patients, P = .0002; and 36% in the wild-type *TP53* versus 0% in the mutated *TP53*-group, P = .01, respectively). Finally, the association between bulky disease and LC was significant (1-year LC was 16% in bulky-patients versus 51% for nonbulky patients, P = .04). Of note, when the characteristics of the GCB and ABC subtype patients were compared, there were no significant differences in tumor stage, tumor size, *TP53* genotype, and chemoresistance between these 2 cohorts, as reported in Table 4.

4 | DISCUSSION

In the present study, we provide evidence that LDRT is effective for palliation of DLBCL, resulting in a well-tolerated and safe regimen. In particular, we showed a 31% CR rate among the 70% of patients with a clinical response. Moreover, the majority of patients (60%) reported a significant amelioration in their global QoL status after LDRT, with a subjective relief of asthenia and pain in 53% of cases.

Despite the large evidence supporting the use of LDRT in indolent NHL patients, there is still a limited number of cases with DLBCL who have been managed with LDRT. The reasons should concern the hypothetical risk of undertreatment of patients, especially in presence of painful and rapidly progressing masses. In this context, the LDRT procedure proposed here shows several advantages. First, this LDRT procedure took 3 days (simulation and delivery), and all patients were planned and treated within 1 week. Second, the LDRT treatment policy allowed the use of radiotherapy multiple times; in this context, 4 of 7 patients not responding to low doses were rescued with additional radiotherapy in standard doses. Third, LDRT offers the chance to rapidly reduce the tumor burden, allowing the use of large radiation fields without significant toxicity, and can be administered in frail patients.

The results shown in the present study support the usefulness of LDRT as an alternative to conventional radiation regimens (total dose to 30-40 Gy) for palliation of DLBCL and strengthen similar findings of previous retrospective studies evaluating LDRT on DLBCL patients.[3,5,6,18] In this context, it is noteworthy that our results in terms of 1-year LC (31%) were in agreement with those reported for conventional radiotherapy in chemoresistant aggressive lymphomas. For example, Aref et al[18,19] reported superimposable results (1-year LC 35%) in a series of patients with chemoresistant DLBCL managed with a radiation dose ≤39.6 Gy. In the present trial, 21 patients (84%) had a chemoresistant disease and 10 (40%) presented a severe decompensation at the time of LDRT. In several cases, LDRT resulted in particularly relevant amelioration of QoL. For example, LDRT to the left lung of 1 woman with stage IVB disease and an extensive lung involvement produced a rapid expansion of the right lung with a significant amelioration of dyspnea (Figure 3).

The exact mechanism of LDRT action is still uncertain, although the low-dose hypersensitivity and the usually rapid responses suggest that activation of cell death mechanisms putatively involving *TP53* should be present.[5,18,20–23] Following this line of reasoning, a nonfunctional *TP53* pathway in the target tissue could predict radioresistance in DLBCL. To investigate this issue, we evaluated *TP53* mutations as a predictive biomarker of LDRT response as a secondary end point. In the current prospective study, the prevalence of *TP53* mutations was comparable with that found in a larger study,[22] and all 2 patients with a crippling mutation of *TP53* did not respond to LDRT (for description of *TP53* mutations, see Table 5). These results, although not statistically significant, are consistent with the findings

TABLE 4 Patients characteristics by DLBCL subtype (N = 19 patients evaluable)

Characteristic	GCB-DLBCL[a]	ABC-DLBCL[b]	*P*
Tumor stage			.26
I-II	3 (100%)	0	
III-IV	9 (56%)	7 (44%)	
Chemoresistance[c]			1
Yes	9 (60%)	6 (40%)	
No	3 (75%)	1 (25%)	
Bulky disease[d]			1
Yes	6 (60%)	4 (40%)	
No	6 (67%)	3 (33%)	
TP53 status (13 patients evaluable)			.19
Wild type	7 (64%)	4 (36%)	
Mutated	0	2 (100%)	
Not available	3 (60%)	2 (40%)	

Abbreviation: DLBCL, diffuse large B-cell lymphoma.

[a]Germinal center B-cell subtype.

[b]Activated B-cell subtype.

[c]Chemoresistance was defined as the failure of chemotherapy to achieve a complete or partial response, or as disease relapse after a complete response.

[d]Bulky disease was defined as >5 cm mass.

TABLE 5 *TP53* status of DLBCL mutated patients

	TP53 Mutations[a]						
Case	Status	cDNA Description	Protein Description	Exon Number	Effect	TA Class	SIFT Class
A	Homo/Emi	c.581T>G	p.L194R	6-exon	Missense	Nonfunctional	Deleterious
B	Hetero	c.524G>A	p.R175H	5-exon	Missense	Nonfunctional	Deleterious
	Hetero	c.544 T>C	p.C182R	5-exon	Missense	Partially functional	Deleterious

Abbreviations: DLBCL, diffuse large B-cell lymphoma; TA, transcriptional activity; SIFT, Sorting Tolerant From Intolerant as reported in the IARCT TP53 Database (http://p53.iarc.fr/).

[a]*TP53* mutations according to IAR TP53 Database (http://p53.iarc.fr/).

of Knoops et al,[5] who also treated DLBCL patients with LDRT. On the other hand, here, the evaluation of the *TP53* status alone was not able to predict response to LDRT. In this context, a limitation could be represented by the availability of pathologic specimens of the target lesion for the evaluation of *TP53* status; in particular, DNA from only 14 of 23 patients could be assayed, making the NGS analysis results largely speculative. Although there was a trend toward the association of mutated *TP53* with resistance to LDRT (P = .12), further studies in larger cohorts are needed.

The tumor subtype classification has been proved to be useful for the identification of DLBCL patients with prolonged survival. In particular, the GCB-DLBCL has been shown to achieve a higher cure rate from the current available chemotherapy with respect to ABC-DLBCL.[24,25] In this trial, GCB cases achieved significantly higher response rate and LC than ABC cases. To our knowledge, this is the first study that demonstrates a predictive role of tumor subtype (cell of origin) for response to LDRT and LC in DLBCL. Moreover, when considering other predictors of response, also bulky disease associated with poor response to LDRT, thus confirming previous findings published by others on a series of patients with indolent lymphoma.[9]

5 | CONCLUSION

Low-dose radiotherapy is effective for palliation of patients with DLBCL in terms of both response rate and duration of response. It is worth noting that favorable results were demonstrated concerning the global health status of the patients, and several symptoms and functional items, basing on the QLQ-C30 questionnaire. The unanticipated role of GCB-DLBCL tumor subtype for predicting response to LDRT constitutes the first evidence of a clinical application of DLBCL-subtype characterization in the setting of radiotherapy. The high potential of LDRT in this subset of patients could lead to the development of the first "targeted radiotherapy" for DLBCL.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

C.F. and P.B. designed the research. M.M., A.E., M.S., and C.F. provided the patients. C.F., P.B., V.C., M.D., R.B., S.P., and R.M. participated in data analysis and interpretation. The first draft was written by C.F., P.B., and V.C., with input from all coauthors. V.G., U.T., M.T., and G.F. participated in revising subsequent drafts of the manuscript before submission.

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