Direct-Acting Antivirals as Primary Treatment for Hepatitis C Virus–Associated Indolent Non-Hodgkin Lymphomas: The BArT Study of the Fondazione Italiana Linfomi

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abstract

PURPOSE We prospectively treated patients with hepatitis C virus (HCV)–associated indolent lymphomas with genotype-appropriate direct-acting antivirals (DAAs) with the aim to evaluate virologic and hematologic outcomes. No prospective studies in this setting have been published so far.

METHODS FIL_BArT is a prospective, multicenter, phase II trial that evaluated genotype-appropriate DAAs in untreated HCV-positive patients with indolent lymphomas without criteria for immediate conventional antilymphoma treatment. The primary objective was sustained virologic response, whereas the main secondary objectives were overall response rate of lymphoma and progression-free survival.

RESULTS Forty patients were enrolled, including 27 with marginal zone lymphoma. Median age was 68 years. Extranodal sites were involved in 14 cases (35%). Main genotypes were 1 in 16 patients and 2 in 21 patients. All patients received genotype-guided DAAs: 17 ledipasvir/sofosbuvir, eight sofosbuvir plus ribavirin, and 15 sofosbuvir/velpatasvir. All patients achieved sustained virologic response (100%). DAAs were well tolerated, with only two grade 3-4 adverse events. Overall response rate of lymphoma was 45%, including eight patients (20%) achieving complete response and 10 (25%) partial response, whereas 16 exhibited stable disease and six progressed. With a median follow-up of 37 months, two patients died (3-year overall survival 93%; 95% CI, 74 to 98) and three additional patients progressed, with a 3-year progression-free survival of 76% (95% CI, 57 to 87).

CONCLUSION HCV eradication by DAAs was achieved in 100% of HCV-positive patients with indolent lymphomas not requiring immediate conventional treatment and resulted in non-negligible rate of lymphoma responses. Treatment with DAAs should be considered as the first-line therapy in this setting.

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INTRODUCTION

Hepatitis C virus (HCV) chronic infection has been unequivocally associated with a wide spectrum of B-cell lymphoproliferative disorders, ranging from mixed cryoglobulinemia to overt B-cell non-Hodgkin lymphomas (B-NHL), including both indolent, especially marginal zone lymphomas (MZL), and aggressive subtypes, mainly diffuse large B-cell lymphomas.1

HCV has been estimated as associated with up to 20% of B-NHL cases in Italy, 14% in Japan, and 11% in United States, whereas rates in the remaining European countries are lower (6%).2,3 The proposed pathogenetic model of HCV-associated B-NHL relies on sustained antigen stimulation by HCV chronic infection, which may lead to the progressive acquisition by B-cells of specific immunologic (B-cell receptor [BCR] stereotypy) and genetic lesions (mutations in nuclear factor-kB or NOTCH signaling pathways); this can result initially in an antigen-dependent, and subsequently in an antigen-independent, B-cell lymphoproliferation.4

Nearly 20 years ago, the seminal observation of regression of HCV-positive splenic lymphomas with villous lymphocytes after HCV eradication by interferon-based antiviral therapy provided major support to the effective causative role of this virus in
CONTEXT

Key Objective
The most convincing evidence supporting the etiologic role of hepatitis C virus (HCV) in indolent non-Hodgkin lymphomas was represented by retrospective observations of lymphoma regression after viral eradication by interferon-free direct-acting antivirals (DAAs). To our knowledge, the phase II BArT trial is the first prospective study evaluating genotype-appropriate DAAs as primary treatment in patients with HCV-associated indolent non-Hodgkin lymphomas not requiring immediate conventional therapy, with the aim to assess both virologic and lymphoma responses.

Knowledge Generated
We demonstrated that (1) all patients enrolled achieved sustained virologic response, with negligible toxicity, (2) nearly half of the patients attained an objective response, without differences between marginal zone lymphoma and non–marginal zone lymphoma subtypes, and (3) responses were highly durable.

Relevance (J.W. Friedberg)
The results of BArT study suggest eradication of HCV with DAAs may result in durable lymphoma regression in a subset of patients. Further studies in patients with HCV-related indolent lymphomas not requiring immediate conventional treatment from other geographic areas are warranted.*

META DAs in HCV-Associated Indolent Lymphomas

B-cell lymphomagenesis.5 Subsequently, a wide number of retrospective series and meta-analyses confirmed this finding, with an overall response rate (ORR) reaching up to 75% of cases, including up to 40% of complete responses (CRs), with a strong correlation between virologic and hematologic responses.6,7 However, the antiproliferative effect of interferon could not have been ruled out.

The introduction of novel interferon-free direct-acting antivirals (DAAs) heralded the opportunity to address this issue. The continuous refinement of DAA regimens allowed the achievement of unprecedented rates of sustained virologic response (SVR) regardless of genotype (95%-100%) with excellent tolerability profile.8 Since the approval of DAAs, several case reports9-12 and retrospective case series from different countries13,14 have pointed out consistent rates of lymphoma regression (ORR 66% and CR 23%)14 after HCV eradication with DAAs in HCV-positive patients with B-cell indolent lymphomas, thus strongly supporting the direct etiological role of HCV in lymphomagenesis.15 However, all these reports suffer from the intrinsic limitations of retrospective studies, including wide heterogeneity in histotypes, tumor burden, type of DAAs regimen, no uniformity of response criteria, and short follow-up. In this regard, although eagerly needed, no prospective study has ever been performed in this setting.

In 2016, the Fondazione Italiana Linfomi (FIL) decided to start the prospective, multicenter, phase II BArT (B-cell lymphoma Antiviral Treatment) trial, aimed at investigating the role of interferon-free DAA regimens in HCV-positive patients with indolent B-NHL without the need of immediate conventional antilymphoma treatment.

METHODS

Patients’ Characteristics
The trial included patients age 18 years or over, who were affected by untreated indolent lymphoma and active chronic infection by genotype 1, 2, 3, or 4 HCV (HCV-RNA-positive). The diagnosis of each indolent lymphoma histotype was established by expert hemopathologists belonging to the FIL pathologist panel according to the 2016 WHO Classification.16 Patients diagnosed with the following subtypes were included: MZL (extranodal of mucosa-associated lymphoid tissue [MALT], nodal, or splenic), lymphoplasmacytic lymphoma, small lymphocytic lymphoma, and grade 1-2 follicular lymphoma. Rare cases of CD5-negative low-grade B-cell lymphoma diagnosed by bone marrow (BM) biopsy alone, which did not fulfill sufficient clinical, pathologic, immunophenotypic, and genetic criteria for any WHO-defined low-grade lymphoma entity, were classified as low-grade CD5-negative B-cell lymphomas not otherwise specified (NOS).17,18 and were included as well.

A measurable disease after diagnostic biopsy or quantifiable BM infiltrate (in case of exclusive BM and leukemic disease) was required. Only patients with asymptomatic disease and absence of criteria for immediate conventional treatment (systemic symptoms, tumor size > 7 cm, symptomatic nodal/extranodal masses or symptomatic splenomegaly, serous effusions, and progressive leukemic phase) were included. Main exclusion criteria comprised severe cytopenias (hemoglobin < 9 g/dL, platelet count < 50 x 10^9/L, or absolute neutrophil count < 1 x 10^9/L), evidence of cirrhosis (defined by stiffness > 12 Kpa at fibroscan), and HIV or HBV positivity. All patients provided written informed consent. All patients achieved antiviral treatment.
consent. The complete study Protocol (online only) is available in the Data Supplement (online only).

Trial Protocol
After enrollment, patients received DAA regimen according to genotype. In brief, patients with genotype 1 and 4 received ledipasvir/sofosbuvir (90/400 mg once daily) for 12 (interferon-naive) or 24 weeks (interferon-experienced). Patients with genotype 2 were treated with sofosbuvir (400 mg once daily) plus ribavirin (1,000 mg if < 75 kg, or 1,200 mg if ≥ 75 kg of body weight divided in two daily doses) for 12 weeks. Finally, patients with genotype 3 received ledipasvir/sofosbuvir plus ribavirin for 24 weeks. Because of the approval in the intervening time of the novel pan-genotypic sofosbuvir plus ribavirin for 24 weeks. Finally, patients with genotype 3 received ledipasvir/sofosbuvir regimen (400/100 mg once daily for 12 weeks). A substantial amendment introduced this regimen for patients with genotype 2 and 3, starting from July 2017.

Oversight
This multicenter, phase II, prospective trial was carried out in agreement with the tenets of the Declaration of Helsinki of 1964, as revised in 2000. The protocol was approved by institutional review boards of each participating FIL center (identification number: 3,730 of February 4, 2016, Ethics Committee of Pavia, coordinating center). Data were collected by the local investigators and were analyzed by the FIL data center. Gilead Sciences supplied the study drugs and partially funded the trial but did not contribute to the study design, conduction, data collection or analysis, and writing of the manuscript. The trial was registered on ClinicalTrials.gov (identifier: NCT02836925).

Staging, Monitoring and End Points
At the time of inclusion, the disease characteristics of enrolled patients were assessed by means of physical examination, complete standard biologic and virologic analyses, BM biopsy, and liver fibroscan. Staging of lymphoma was performed by means of total-body contrast-enhancement computed tomography (CT) scan, whereas positron emission tomography scan for 18-fluorodeoxyglucose-avid histologies was optional. SVR was defined as the clearance of HCV-RNA after 12 weeks from the completion of DAAs. Lymphoma response was assessed according to the Lugano criteria by CT after 12 weeks from completion of DAAs. Additional details about staging, response evaluation, and monitoring are available in the Data Supplement.

At the time of the study design, no reliable estimation of the expected ORR induced by DAAs in patients with HCV-positive indolent B-NHL was available. For this reason, we selected a virologic primary end point, that is, SVR at 12 weeks after the completion of DAAs. Main secondary end points were ORR of lymphoma, progression-free survival (PFS), duration of response (DOR), overall survival (OS), and toxicity according to Common Terminology Criteria for Adverse Events v.4.03 (see the Data Supplement for the complete list of secondary end points).

Sample Size and Statistical Analysis
The sample size was calculated at the primary end point, that is, SVR, taking into account SVR rates after interferon-based regimens ranging between 40% and 70% and after novel DAA regimens ranging between 94% and 99%. The study was designed according to Fleming A’Hern’s single-stage phase II design, setting the null hypothesis (p0, unacceptably low proportion of SVR) as equal to 0.80, the alternative hypothesis (p1, minimum required proportion of SVR for DAAs regimens) equal to 0.95, and taking into account an α error of 0.05 and a power of 0.90. According to these assumptions, the required sample size resulted equal to 44 patients, and the minimum number of SVR to exclude the null hypothesis was 40. Accordingly, accrual was interrupted when 40 SVRs were achieved.

Continuous covariates were summarized with median and range, whereas categorical variables were summarized as absolute and percentage frequencies. The association between categorical variables between groups was evaluated by means of Fisher’s exact test. The Kaplan-Meier product limit method was used to estimate PFS, DOR, and OS (see the Data Supplement for statistical details). The study was not powered for subanalyses and the tests were not corrected for multiple testing comparisons. All computations were carried out using Stata 17.0 (StataCorp LLC, College Station, TX).

RESULTS
Patients
From May 18, 2016, to July 29, 2019, 40 HCV-RNA-positive patients with indolent lymphoma were enrolled at 13 FIL sites and underwent DAA treatment as mandated by the protocol (Fig 1). The baseline hematologic and virologic characteristics of the study population are summarized in Table 1. Most patients were diagnosed with MZL (27 patients, 68%), including 14 extranodal of MALT, seven nodal marginal zone lymphoma (NMZL), and six splenic marginal zone lymphoma cases. Among the remaining 13 non-MZL cases, six patients had lymphoplasmacytic lymphoma, four low-grade CD5-negative B-cell lymphoma NOS, two small lymphocytic lymphoma, and one follicular lymphoma. All patients were Caucasian. Median age was 68 years (range, 45-83 years). Stage was III or IV in 34 patients (85%). Extranodal sites were involved in 14 patients (35%), including ocular adnexa in six, skin/soft tissue in five, muscles in two, salivary glands in two, and breast, nasopharynx, and liver in one case each. BM was involved by lymphoma in 24 patients (60%), whereas 10 (25%) showed splenomegaly (defined by longitudinal diameter longer than 13 cm). HCV genotype was 1 in 16 patients (40%), 2 in 21 patients (53%), 3 in two patients (5%), and 4 in one patient (2%). Cryoglobulins were detected in 14
patients (35%), with a median cryocrit level of 3% (range, 1%-10%). Rheumatoid factor was increased in 35% of patients, whereas C3 and C4 levels were reduced in 18% and 41%, respectively. Cirrhosis was excluded in all cases by fibroscan (median liver stiffness 6.4 KpA; range, 2.8-11.6 KpA).

### Antiviral Treatment

Four patients (10%) with genotype 1 or 4 had previously failed an interferon-based antiviral regimen and underwent a 24-week course of ledipasvir/sofosbuvir, whereas 13 interferon-naive genotype 1 patients received the standard 12-week ledipasvir/sofosbuvir regimen (Fig 1). Before the introduction of the substantial amendment, eight patients with genotype 2 were treated with sofosbuvir plus ribavirin for 12 weeks. Subsequently, 13 genotype 2 and two genotype 3 patients received the novel pan-genotypic sofosbuvir/velpatasvir regimen for 12 weeks. There was no major protocol violation related to the experimental treatment, and all patients completed the scheduled treatment regimen, without any drug reduction or interruption.

### Efficacy

The primary end point was met as the 40 enrolled patients achieved HCV eradication at the 12-week evaluation after the completion of DAAs (SVR 100%). Pertaining to on-treatment virologic responses, after 4 weeks of therapy, 37 of 40 treated patients cleared HCV-RNA (92.5%), whereas at the end of treatment, HCV-RNA was undetectable in all

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**FIG 1.** Eligibility, diagnosis, treatment, responses, and follow-up of the 40 patients with hepatitis C virus–positive indolent lymphomas treated with interferon-free DAAs. CR, complete response; DAA, direct-acting antiviral; FL, follicular lymphomas; GT, genotype; IFN, interferon; LDV, ledipasvir; LPL, lymphoplasmacytic lymphomas; MZL, marginal zone lymphoma; NOS, not otherwise specified; PR, partial response; RBV, ribavirin; SD, stable disease; SLL, small lymphocytic lymphomas; SOF, sofosbuvir; SVR, sustained virologic response; VEL, velpatasvir.
TABLE 1. Demographics and Clinical Characteristics of 40 Patients With HCV-Positive Indolent Lymphomas Treated With Direct-Acting Antivirals

| Characteristic                          | No. (%) |
|----------------------------------------|---------|
| Age, years, median (range)             | 68 (45-83) |
| Age, years, < 60/≥60                   | 12/28 (30/70) |
| Male/female                            | 17/23 (42/58) |
| Marginal zone lymphoma                 | 27 (68) |
| Splenic                                 | 6 (15) |
| Nodal                                   | 7 (18) |
| Extranodal                              | 14 (35) |
| Low-grade CD5-negative B-cell lymphoma NOS | 4 (10) |
| SLL                                     | 2 (5) |
| LPL                                     | 6 (15) |
| FL                                      | 1 (2) |
| Advanced stage (III-IV)                | 34 (85) |
| Nodal involvement                      | 24 (60) |
| Extramedial involvement†               | 14 (35) |
| Spleen involvement                     | 10 (25) |
| BM involvement                         | 24 (60) |
| ECOG PS                                 | 37 (92) |
| 0                                       | 3 (8) |
| Hemoglobin < 12 g/dL                   | 7 (18) |
| Platelets < 100 × 10³/L                | 5 (13) |
| ANC < 1.5 × 10⁹/L                      | 1 (2) |
| ALC > 5 × 10⁹/L                        | 3 (8) |
| LDH > ULN                              | 13 (32) |
| Monoclonal component                   | 15 (38) |
| Albumin < 3.5 g/dL                     | 3 (7) |
| HCV genotype                           |         |
| 1                                       | 16 (40) |
| 2                                       | 21 (53) |
| 3                                       | 2 (5) |
| 4                                       | 1 (2) |
| HbAb+                                   | 11 (28) |
| Cryoglobulins                          | 14 (35) |
| Rheumatoid factor > 20 IU/mL³          | 11 (35) |
| C3 levels < 80 mg/dL                   | 6 (18) |
| C4 levels < 10 mg/dL                   | 13 (41) |
| Liver stiffness, kPa, median (range)    | 6.4 (2.8-11.6) |
| Previous IFN-based antiviral therapy    | 4 (10) |

NOTE. Data are No. (%) unless otherwise indicated. Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; BM, bone marrow; ECOG PS, Eastern Cooperative Oncology Group performance status; FL, follicular lymphoma; HbAb, hepatitis B core antibody; HCV, hepatitis C virus; IFN, interferon; LDH, lactate dehydrogenase; LPL, lymphoplasmacytic lymphoma; NOS, not otherwise specified; SLL, small lymphocytic lymphoma; ULN, upper level of normal.

†BM and spleen were not considered as extranodal sites.

Data available in 31 cases.

Data available in 33 cases.

Data available in 32 cases.

but two cases (95%). No HCV-RNA breakthrough or virologic relapse was detected (Fig 2A). Overall, eight of 14 patients (57%) cleared cryoglobulins (complete immunologic response) and one (7%) reduced cryocrit > 50% (partial immunologic response).

At the lymphoma restaging, eight patients (20%) achieved a CR and 10 (25%) a partial response (PR), with an ORR of lymphoma of 45% according to CT-based Lugano criteria. By contrast, 16 patients (40%) exhibited stable disease (SD) and six (15%) experienced progressive disease (Table 2, Fig 2B). Overall, no significant difference in ORR was recorded between MZL and non-MZL cases (48 v 38%, P = .74). Among MZL, the best ORR was achieved by patients with MALT MZL (71%), followed by NMZL (43%), whereas none of the six patients with splenic marginal zone lymphomas obtained a response according to Lugano criteria (P = .014). Notably, despite the small sample size, responses were detected across all non-MZL subtypes, including one CR (8%) in a patient with low-grade CD5-negative B-cell lymphoma NOS, and four PR (30%). Interestingly, response rate varied among the different organs involved: eight of 24 patients (33%) demonstrated a ≥ 50% decrease in lymph node size, including five patients with CR of lymphadenopathies (21%). Overall, 15 of 24 patients (62.5%) experienced some extent of nodal tumor size reduction (Data Supplement). Ten of 14 patients (71%) showed a ≥ 50% reduction in extranodal lesions, including six patients with complete regression of lymphoma in extranodal sites (43%). Three of 10 patients (30%) with splenomegaly normalized spleen size. Finally, six of 19 patients (32%) with BM involvement and available BM biopsy at restaging cleared BM infiltrate.

No significant differences in ORR or CR rates (P = .226 and P = .272, respectively) were recorded according to the three different DAA regimens used (ledipasvir/sofosbuvir, sofosbuvir plus ribavirin, or sofosbuvir/velpatasvir). Similarly, there was no difference in terms of ORR between patients with genotype 2 (11/21, 52%) and those with other genotypes (7/19, 37%; P = .360). Considering 14 patients with circulating cryoglobulins at baseline, ORR was 75% (6/8) among complete immunologic responders compared with 33% (2/6) among partial or null immunologic responders (P = .562).

At univariate analysis, only splenomegaly (OR 2.98; 95% CI, 1.17 to 7.58; P = .023) and absolute lymphocyte count (OR 50%, P = .032) were significantly associated with probability of nonresponse (Data Supplement).

Safety

All DAA regimens were well tolerated, and no patient discontinued or reduced the dose of study drugs because of adverse events (AEs). Notably, no clinically significant drug interactions were observed in the study. A total of 32 AEs were registered in 17 patients (43%), including 30 grade
FIG 2. (A) RVR (at 4 weeks of therapy), ETR, SVR (at 12 weeks from the end of the treatment), and virologic relapse rates in 40 enrolled patients treated with DAAs. (B) ORR (calculated as CR plus PR) among 40 patients with hepatitis C virus–positive indolent lymphoma who received interferon-free DAAs, as well response among the patients with SD or PD. The patients were evaluated according to the two main disease cohorts: MZL and other indolent lymphoma histologies (non-MZL). The numbers in parentheses indicate the number of patients who had the specified response. (C) OS of the 40 enrolled patients. (D) PFS of the 40 enrolled patients. (E) PFS in 40 enrolled patients according to histotype group (MZL v non-MZL). (F) DOR in 18 patients with CR or PR of lymphoma after interferon-free DAAs. (G) Swimmer plot of DOR in 18 patients with CR or PR of lymphoma after interferon-free DAAs. CR, complete response; DAA, direct-acting antiviral; DOR, duration of response; ETR, end-of-treatment response; MZL, marginal zone lymphoma; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; RVR, rapid virologic response; SD, stable disease; SVR, sustained virologic response.
1-2 and two grade 3-4 AEs (Table 3). Only one serious AE (grade 3 infiltrating breast cancer) was registered in a 76-year-old female patient and was considered unrelated to study drugs. One patient receiving ledipasvir/sofosbuvir experienced a transient grade 4 asymptomatic lipase increase, which resolved spontaneously after therapy completion. Overall, the most common AE was fatigue (20%). Hematologic toxicity of DAA regimens was mild, as only four grade 1 AEs were reported, namely one case of thrombocytopenia and three cases of decreased hemoglobin, two of which occurred in patients receiving ribavirin.

Outcome
At the data cutoff (January 15, 2022), with a median follow-up of 37 months from inclusion (95% CI, 28 to 42), two patients died after 10 months (lymphoma progression) and 35 months (cause unrelated to disease) from the start of treatment (3-year OS, 93%; 95% CI, 74 to 98; Fig 2C). After restaging, three patients progressed (one exhibiting SD and two PR after DAAs; Data Supplement). The median PFS for all patients was not reached, whereas the 3-year PFS was 76% (95% CI, 57 to 87; Fig 2D). According to histotype group (non-MZL v MZL), the 3-year PFS was 77% (95% CI, 44 to 92) versus 73% (95% CI, 47 to 88; P = .678; Fig 2E; Data Supplement). At univariate analysis, only male sex (HR, 3.97; 95% CI, 1.02 to 15.4; P = .046) and white blood cell count (HR 2.24; 95% CI, 1.04 to 4.83; P = .039) were significantly associated with higher probability of lymphoma progression (Data Supplement).

Considering the 18 responding patients, the 3-year rate of DOR was 89% (95% CI, 62 to 97; Figs 2F-2G; Data Supplement), with the median duration of CR and PR being 22 and 23 months, respectively. Interestingly, only one of 16 patients (6%) who displayed SD after DAAs experienced disease progression during the follow-up (median, 37 months).

All the nine patients who exhibited lymphoma progression initiated a conventional treatment (one involved-site radiotherapy, six rituximab plus bendamustine, and two rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone). At the last follow-up, seven of nine patients were in CR, one was in progression after multiple lines of therapy (NMZL), and one died after two lines of therapy (low-grade CD5-negative B-cell lymphoma NOS). Notably, no patient experienced histologic transformation into high-grade lymphoma.

DISCUSSION
Similar to what was at first evidenced in gastric MALT lymphomas after Helicobacter pylori eradication by antibiotics,23 the regression of a wide spectrum of HCV-associated indolent lymphomas after viral clearance
through DAAs unequivocally demonstrates the role of HCV in lymphomagenesis, most likely reflecting a similar indirect pathogenetic mechanism relying on continuous antigen-driven stimulation.4 To the best of our knowledge, the BArT study is the first prospective clinical trial investigating the antiviral and antitumor activity of DAAs as primary treatment in HCV-associated indolent lymphomas. In this specific setting, all the evaluated sofosbuvir-based DAA combinations exhibited an excellent rate of success in HCV eradication (100%) with negligible toxicity. Notably, in the majority of patients (92.5%), HCV-RNA was cleared only after 4 weeks of therapy. More importantly, after almost 2 decades of retrospective clinical observations reporting lymphoma regression after antiviral therapy with either interferon-based5,7 or, more recently, interferon-free DAA-based combinations,13,14 we reliably demonstrated that the viral clearance achieved through HCV-directed therapy is able per se to induce tumor regression in 45% of HCV-positive patients with indolent lymphomas. Indeed, contrary to interferon-based antiviral therapy adopted in the past, the achievement of lymphoma responses after DAAs should have been mediated exclusively by their potent antiviral activity leading to rapid HCV eradication and consequent cessation of antigen-driven stimulation.4 In this regard, in comparison with our study, the apparently higher rates of lymphoma responses reported by retrospective series evaluating interferon-based antiviral therapy (ORR 73%6,7 v 45%) even with clearly inferior SVR rates (46%-76%7,24 v 100%) could be largely explained by the well-known antiproliferative activity of interferon in lymphoproliferative disorders.

Considering the novel interferon-free DAAs regimens, the ORR evidenced by this prospective study seems to be slightly inferior with respect to what was reported by a recent large, retrospective, international study in a comparable population (ORR 69% in 66 patients), although CR rates appear substantially superimposable (20% v 21%).14 This discrepancy may be related to different case selection (histotypes, stage, and tumor burden) as well as more accurate evaluation of response in the present prospective trial, given that another 30% of patients had some extent of reduction of nodal sizes without reaching PR criteria. Given that the majority of enrolled patients were affected by MZL and the study was not powered to detect subgroup differences, the comparison of ORR and CR rates across different histotypes should be interpreted with caution. However, it is of significance that a higher proportion of responses were detected at the level of extranodal localizations (71%), especially at sites typically involved in HCV-positive MALT lymphomas, such as skin, ocular adnexa, and salivary glands.25 Conversely, splenic involvement and high lymphocyte counts were associated with lower responsiveness to DAAs, possibly accounting for a more systemically spread and antigen-independent disease. However, despite the limitation of the low number of patients, the similar rate of responses achieved in a wide

### TABLE 3. Adverse Events in All 40 Patients With Hepatitis C Virus–Positive Indolent Lymphomas Treated With Direct-Acting Antivirals

| Adverse Event                      | Grade | Total, No. (%) | LDV/SOF (n = 17), No. | SOF Plus RBV (n = 8), No. | SOF/VEL (n = 15), No. |
|-----------------------------------|-------|----------------|-----------------------|--------------------------|-----------------------|
| Any AE                            | 1     | 26             | 32                    | 8                        | 11                    |
| Hematologic AEs                   | 2     | 4              | 0                     | 0                        | 2                     |
| Neutropenia                       | 3     | 0              | 0                     | 0                        | 0                     |
| Thrombocytopenia                  | 4     | 0              | 0                     | 0                        | 0                     |
| Extrahematologic AEs              | 5     | 22             | 28                    | 8                        | 11                    |
| GI                                | 6     | 1              | 2 (5.0)               | 0                        | 0                     |
| Fatigue                           | 7     | 9              | 3 (7.5)               | 1                        | 0                     |
| Metabolism and nutrition          | 8     | 3              | 3 (7.5)               | 1                        | 0                     |
| Musculoskeletal and connective tissue | 9     | 1              | 1 (2.5)               | 0                        | 1                     |
| Nervous system                    | 10    | 3              | 3 (7.5)               | 0                        | 2                     |
| Psychiatric                       | 11    | 1              | 1 (2.5)               | 0                        | 0                     |
| Renal and urinary                 | 12    | 1              | 1 (2.5)               | 1                        | 0                     |
| Respiratory/thoracic              | 13    | 1              | 2 (5.0)               | 0                        | 1                     |
| Skin and subcutaneous tissue      | 14    | 2              | 4 (10.0)              | 1                        | 2                     |
| Neoplasms                         | 15    | 0              | 0                     | 0                        | 0                     |
| Lipase increase                   | 16    | 0              | 0                     | 0                        | 0                     |

**Abbreviations:** AE, adverse event; LDV, ledipasvir; RBV, ribavirin; SOF, sofosbuvir; VEL, velpatasvir.
variety of MZL and non-MZL cases may suggest the presence of more comprehensive immunogenetic mechanisms of lymphoma response to viral eradication upon DAA. In this regard, two recent studies showed that 54%-70% of HCV-positive lymphoproliferative diseases display stereotyped light-chain complementarity determining region 3, which are enriched in sequences homologous to anti-HCV E2 antibodies. 🌐 Intriguingly, these patients harbored mutations more frequently in nuclear factor-kB, NOTCH, or BCR signaling pathways’ genes and exhibited a significantly higher probability of hematologic response after DAA with respect to nonstereotyped cases, pointing out a possible link between specific stereotyped BCR, acquisition of oncogenic lesions, and responsiveness to HCV eradication.

With regard to the outcome, with a median follow-up of 37 months, lymphoma responses after DAA were stable and durable (3-year DOR 89%), which was also the case for patients achieving PR. Moreover, patients exhibiting SD also showed a favorable prognosis, as only one of 16 patients ultimately experienced lymphoma progression. 🌐 Notably, the estimated 3-year PFS appears similar to the one reported in the previously cited retrospective study (76% v 79%14), and compares favorably with a retrospective cohort of 100 patients treated with first-line interferon-based antiviral therapy (74%). 🎯 Finally, viral eradication may exert favorable influence also in relation to postprogression outcome, possibly because of better treatment tolerability or cessation of mutational pressure and reduction of secondary genetic lesions in lymphoma cells. In this regard, at the last follow-up, eight of the nine patients experiencing disease progression after DAA were alive, of whom seven exhibited an ongoing CR.

Although these results seem to be extremely promising, the lack of a control untreated population makes it obviously difficult to dissect the precise extent of the benefit associated with HCV eradication by DAA on the disease outcome. Of note, in the interferon era, two independent large retrospective observational studies were able to demonstrate a survival advantage in patients with HCV-associated indolent lymphomas treated with antiviral therapy at any time during their disease history. 📈 Moreover, in both series, PFS was also significantly shorter in patients who did not receive any antiviral therapy. 🎯 In particular, the Italian study reported a 5-year PFS of 45% in this subgroup with respect to 63% of patients treated with antiviral therapy as primary treatment. 🌐 Finally, a previous retrospective comparative analysis pointed out a similar OS and PFS among matched cases treated with interferon-free and interferon-based antiviral therapy. 🚀 Overall, in the light of these indirect comparisons, the prospective results of the BArlt study support with a higher level of evidence the recommendation that patients with HCV-associated indolent lymphomas should receive modern DAA-based antiviral therapy as first-line treatment, given their absolute efficacy in HCV eradication, excellent tolerability, and ultimate favorable influence on the disease outcome. It has to be remembered, however, that the population of this study did not include patients with symptomatic or high-tumor-burden indolent lymphomas with definite indication for prompt treatment initiation. However, in the clinical practice, it could be assumed that the majority of such patients could receive DAA immediately before the initiation of immunochemotherapy with a delay of only 8-12 weeks.

The different DAA regimens used in this trial reflect the rapid evolution of antiviral therapy toward pan-genotypic ribavirin-free regimens, such as sofosbuvir/velpatasvir, which reached an efficacy close to 100% in terms of SVR in all subgroups, including genotypes 5 or 629 and those with different ethnicities. 🌐 For this reason, although all enrolled patients came exclusively from Italy, we believe that the results of our study may be substantially applicable to patients with different ethnicity or geographical origin, although only prospective or real-life studies conducted in different countries may definitely highlight this issue. Finally, given the excellent tolerability demonstrated by DAA in this setting, future studies may evaluate these regimens in combination with anti-CD20 monoclonal antibodies and/or novel agents (ie, Bruton’s tyrosin-kinase and phosphatidylinositol 3-kinase inhibitors) as primary treatment in patients with high-tumor-burden HCV-related indolent lymphomas, especially MZL. Few preliminary experiences exploring the combination of DAA and rituximab-based immunochemotherapy or novel agents in HCV-positive patients with aggressive lymphomas have been reported without evidence of significant drug-drug interactions. 📈

In conclusion, our prospective data strongly support the recommendation that patients with HCV-positive indolent lymphoma without need of immediate treatment should receive DAA as the first-line therapy with the aims of eradicating the virus and achieving durable lymphoma regression.

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DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Direct-Acting Antivirals as Primary Treatment for Hepatitis C Virus–Associated Indolent Non-Hodgkin Lymphomas: The BArT Study of the Fondazione Italiana Linfomi

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