REGULAR ARTICLE

blood advances

Carfilzomib combined with rituximab, ifosfamide, carboplatin, and etoposide for relapsed or refractory DLBCL

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Key Points

- Addition of carfilzomib to R-ICE is well tolerated in patients with relapsed/refractory DLBCL.
- Patients with non-GCB DLBCL benefit significantly from C-R-ICE with an ORR of 85%.

The CORAL study highlighted the need to develop novel salvage regimens in relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) previously treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. Carfilzomib (CFZ) can overcome rituximab chemotherapy resistance in lymphoma preclinical models by targeting the ubiquitin-proteasome system. We conducted an investigator initiated, single-center, open-label, prospective phase 1 study evaluating the safety and efficacy of CFZ in combination with rituximab, ifosfamide, carboplatin, and etoposide (C-R-ICE) in high-dose chemotherapy with autologous stem cell transplant (HDC-ASCT) eligible patients with R/R DLBCL (NCT01959698). In the dose-escalation phase, 18 patients were enrolled at 6 dose levels with no dose-limiting toxicities noted. CFZ 45 mg/m^2 was selected as the recommended dose for expansion. Eleven additional patients were enrolled in the dose-expansion phase. Overall response rate (ORR) was 66% (48% CR; 17% PR); 52% patients underwent HDC-ASCT. An ORR of 85% was observed in patients with nongerminal center B-cell–like (non-GCB) DLBCL compared with only 13% in those with GCB DLBCL. Median progression-free survival (PFS) was 15.2 months (5.1 months, not reached [NR]), and median overall survival (OS) was 22.6 months (6.8 months, NR). Patients with non-GCB subtype had a significantly longer PFS (NR vs 6.6 months; P = .0001) and OS (NR vs 6.6 months; P = .001) than those with GCB subtype. C-R-ICE is well tolerated in patients with R/R DLBCL with toxicities comparable to rituximab, ifosfamide, carboplatin, and etoposide therapy. Our data show that patients with non-GCB DLBCL benefit significantly from incorporating CFZ into second-line therapy and HDC-ASCT.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL), comprising 30% to 35% of all NHLs.[1] Although upfront chemoimmunotherapy can cure most cases, about 30% of patients with DLBCL develop relapsed/refractory (R/R) disease.[2,3] The outcome of

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The full-text version of this article contains a data supplement.

patients with R/R DLBCL is poor, with only 46% eligible for high-dose chemotherapy and autologous stem cell transplant (HDC-ASCT) and 12% of ineligible patients achieving long-term event-free survival.[4] The PARMA study established the role of HDC-ASCT in consolidation treatment for patients with R/R DLBCL responding to second-line chemotherapy; however, current standard salvage chemotherapy regimens (eg, R-ICE [rituximab, ifosfamide, carboplatin, and etoposide], R-DHAP [rituximab, dexamethasone, cytarabine, and cisplatin], R-ESHAP [rituximab, etoposide, cytarabine, cisplatin, and methylprednisolone], R-GDP [rituximab, gemcitabine, dexamethasone, and cisplatin]) used before HDC-ASCT in the post rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) era yield suboptimal outcomes.[5] The overall response rate (ORR) and complete response (CR) rate after salvage therapy with R-ICE or R-DHAP is 63% and 38%, respectively.[6,7] Moreover, the 3-year progression-free survival (PFS) of patients with R/R DLBCL initially treated with R-CHOP and then with salvage R-ICE or R-DHAP and HDC-ASCT is only 30%. Depth of remission to salvage chemotherapy is independently prognostic of post–HDC-ASCT outcomes.[8] Incorporating active novel agents into the management of R/R DLBCL may improve these clinical outcomes.

The ubiquitin-proteasome system plays an important role in acquiring resistance to rituximab and chemotherapy agents in B-cell lymphoma.[9,10] Carfilzomib (CFZ, PR-171) is a potent, tetrapeptide ketoepoxide–based inhibitor specific for the chymotrypsin-like active site of the 20S proteasome. It was first approved for the treatment of R/R multiple myeloma in 2012 and has since become well established in the therapeutic landscape of myeloma.[11] In preclinical R/R DLBCL models, CFZ can overcome resistance to chemotherapeutic agents, upregulate proapoptotic proteins, and cause dose- and time-dependent cytotoxicity.[12] We hypothesized that targeting the ubiquitin-proteasome system with novel proteasome inhibitors such as CFZ will be tolerable and result in higher overall and complete remission rates and improved outcomes after HDC-ASCT in patients with R/R refractory DLBCL. We report the results of a phase 1 study which combines CFZ with standard doses of R-ICE in transplant-eligible patients with R/R DLBCL.

Methods

Patients

Adults (age ≥18 years and ≤75 years) with histologically confirmed R/R CD20 positive DLBCL who had received at least 1 prior rituximab-based immunochemotherapy were eligible. Patients must have been eligible for HDC-ASCT at the time of study enrollment as per institutional criteria. Other aggressive lymphoma histologies (including transformed lymphoma and Richter's transformation) and patients with active central nervous system disease were excluded. Full inclusion and exclusion criteria are presented in the supplemental Data.

The clinical study was performed according to the International Council for Harmonization's good clinical practice guidelines and the ethical principles of the Declaration of Helsinki. The institutional review board approved it. All patients provided written informed consent. This trial was registered at www.clinicaltrials.gov as #NCT01959698.

Study design and treatment

This investigator initiated, phase 1/1b, open-label, 2-part (dose escalation [part 1] and dose-expansion [part 2]) study of CFZ in combination with the R-ICE regimen in R/R DLBCL was conducted at Roswell Park Comprehensive Cancer Center (enrollment from May 2014 to March 2020). The primary objectives of part 1 were to evaluate the safety and tolerability of CFZ in combination with R-ICE and determine the maximum tolerated dose and recommended dose for expansion. The primary objectives for part 2 were to evaluate safety and tolerability at the recommended dose. Secondary objectives included preliminary evaluation of activity, the feasibility of successful mobilization of autologous stem cells, and study of differences in clinical outcomes between germinal center B-cell–like (GCB) and non-GCB subtypes. Exploratory assessments included a correlation of proteasomal inhibition with baseline characteristics and outcomes.

Patients received CFZ (at 6 dose levels) on days 1, 2, 8, 9, and standard doses of rituximab-ICE on days 3 to 6 (Figure 1). Cycles were repeated every 21 days for a maximum of 3 cycles before HDC-ASCT.

In part 1, patients were assigned doses using a 3 + 3 dose-escalation design, overseen by a dose-escalation steering committee. No intrapatient dose escalation was permitted. In part 2, patients were assigned to the recommended dose level identified in part 1. Detailed treatment administration is described in the supplemental Data.

Toxicity was assessed using National Cancer Institute common terminology criteria for adverse events (AEs) version 4.0. Dose-limiting toxicities were defined as described in the supplemental

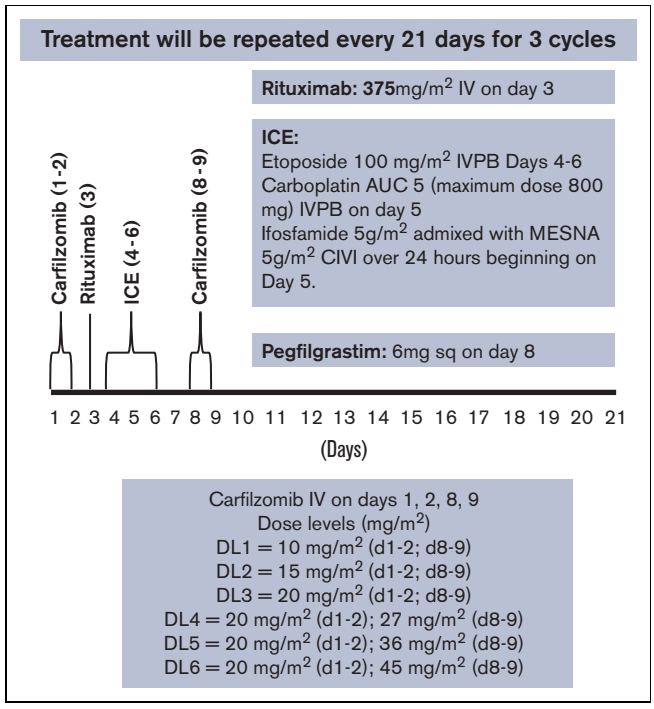

Figure 1. Treatment schema and dose levels. AUC, area under curve; CIVI, continuous intravenous infusion; DL, dose level; IV, intravenous; IVPB, intravenous piggy bag; MESNA, sodium 2-mercaptoethane sulfonate.

Data during cycle 1 in part 1 (dose-limiting toxicity observation period) except when events were clearly because of underlying disease or extraneous causes. Based on the 3 + 3 design, the maximum tolerated dose was the highest dose level at which 0 of the first 3 patients treated or ≤1 of the first 6 patients treated had a dose-limiting toxicity during cycle 1 of part 1.

Assessments

Safety assessments included AEs, serious AEs, dose-limiting toxicities, periodic 12-lead electrocardiograms, physical examinations, vital signs, Eastern Cooperative Oncology Group performance status, and laboratory tests (hematology, coagulation panel, biochemistry, and pregnancy testing [in women of childbearing potential]).

Antitumor activity measures were ORR, duration of response, overall survival (OS), and PFS. Imaging, laboratory, and pathological studies were conducted for staging and response evaluation at baseline between cycle 2 day 15 and cycle 2 day 28 and at the end of study evaluation after Cycle 3. Investigators adjudicated patients' responses to treatment as CR, partial response (PR), stable disease, or progressive disease according to the revised International Working Group response criteria.[13]

Blood samples for pharmacokinetic analysis were collected as described in supplemental Data. CFZ concentrations in plasma were determined using a validated liquid chromatography/mass spectrometry/mass spectrometry method in Roswell Park Comprehensive Cancer Center's bioanalytics, metabolomics and pharmacokinetics core facility. An assay for proteasome inhibition in whole blood plasma samples and peripheral blood mononuclear cells was performed using an enzymatic assay for chymotrypsin-like activity, and the degree of proteasomal inhibition was correlated with response and baseline patient and disease characteristics. The samples were taken on day 1 of cycle 1, predose and 1 hour post end of infusion; day 2 of cycle 1, predose and 1 hour after the end of infusion and day 3, cycle 1, before rituximab administration.

Statistical analysis

The basic design of the phase 1 trial portion of the trial utilized a standard 3 + 3 dose-finding scheme with 6 dose levels, DL1 to DL6, as per Figure 1. The trial was initially designed to be a phase 1/2 trial. The sample size calculation for the original phase 2 population was based on testing the hypotheses concerning the proportion of the population which experienced an objective response rate ORR of either a CR or PR. It was estimated that a total of 37 patients were required to achieve ~80.1% power to detect differences of 20% points (50% vs 70%) at $\alpha = 0.05$ based on historical R-ICE data. Because of budget constraints, the trial was modified to a phase 1/1b trial to enroll a maximum of n = 30 patients, in which 1b corresponded to the expansion cohort. The statistical plan was modified to summarize the ORR as the sample percentage and the corresponding exact 95% confidence interval (CI) for all evaluable patients. Continuous descriptive data were summarized as means and standard deviations. Categorical data were summarized with a sample percentage. PFS and OS curves were estimated using the Kaplan-Meier estimator. A swimmer's plot was generated to illustrate patient disposition. Associations among categorical variables were tested using an exact χ^2 test.

Results

Patient disposition and characteristics

Patient and disease characteristics are described in Table 1. A total of 29 patients were included in the study, 18 in the dose-escalation phase and 11 in the dose-expansion phase (supplemental Figure 1). The median age was 62 years (range, 29-75 years) with 62% of patients aged ≥60 years, 55% were male, 83% had Karnofsky performance score >70, 59% had stage IV disease, 28% had GCB subtype, and 69% had non-GCB subtype DLBCL by Hans algorithm. MYC and BCL2/BCL6 rearrangements (double-hit lymphoma) were noted in 3 patients; an additional 2 patients had an isolated MYC rearrangement. The median time from diagnosis to relapse was 15.9 months (range, 2.2-102.1 months), with 31% patients having primary refractory disease. Median number of prior lines of therapy was one with majority of patients receiving R-CHOP in the frontline setting (59%).

Table 1. Demographic characteristics

	n = 29, n (%)
Age (y)	62 (29-75)
Age groups	
<60	11 (38)
60-69	14 (48)
70 and above	4 (14)
Gender	
Male	16 (55)
Female	13 (45)
Race	
White	29 (100)
KPS at diagnosis	
≤70	4 (14)
>70	24 (83)
Missing	1 (3)
Extranodal disease at diagnosis	
0-1	20 (69)
≥2	7 (24)
Missing	2 (7)
Bone marrow involvement at diagnosis	
Yes	5 (17)
No	16 (55)
Missing	8 (28)
Stage at diagnosis	
I	2 (7)
II	2 (7)
III	6 (21)
IV	17 (59)
Missing	2 (7)

KPS, Karnofsky Performance Scale, IPI- International Prognostic Index; R-EPOCH, rituximab-etoposide-prednisone-vincristine-cyclophosphamide-doxorubicin, R-HyperCVAD, rituximab-hyper fractionated cyclophosphamide-vincristine-doxorubicin-dexamethasone-methotrexate-cytarabine, R-DHAC-rituximab-dexamethasone-cytarabine-carboplatin.

*Patient had primary mediastinal B-cell lymphoma.

†Total exceeds 100% as some patients received 2 lines of therapy.

Table 1 (continued)

	n = 29, n (%)
IPI risk score at diagnosis (original)	
Low (0-1)	6 (21)
Low-intermediate (2)	5 (17)
High-intermediate (3)	9 (31)
High (4-5)	5 (17)
Missing	4 (14)
IPI risk group (dichotomous)	
Low (0-2)	11 (38)
High (3-5)	14 (48)
Missing	4 (14)
Cell of origin (by Hans algorithm)	
GCB	8 (28)
Non-GCB	20 (69)
Unclassified*	1 (3)
Double expressor status	
Yes	7 (35)
No	13 (65)
Missing	9
Cytogenetics	
MYC rearranged (sole abnormality)	2
MYC and BCL2 rearranged	1
MYC and BCL6 rearranged	2
MYC, BCL2 and BCL6 rearranged	0
Double hit lymphoma	
Yes	3 (10)
No	26 (90)
Median time from diagnosis (mo)	
Primary refractory disease	15.9 (2-102)
Yes	9 (31)
No	20 (69)
Relapse within 12 months of diagnosis	
Yes	11 (38)
No	18 (62)
Median no. of prior therapies	
1	1 (1-2)
2	27 (93)
Prior therapies†	2 (7)
R-CHOP	17 (59)
R-EPOCH	10 (35)
R-HyperCVAD	2 (7)
R-DHAC	2 (7)

KPS, Karnofsky Performance Scale, IPI- International Prognostic Index; R-EPOCH, rituximab-etoposide-prednisone-vincristine-cyclophosphamide-doxorubicin, R-HyperCVAD, rituximab-hyper fractionated cyclophosphamide-vincristine-doxorubicin-dexamethasone-methotrexate-cytarabine, R-DHAC-rituximab-dexamethasone-cytarabine-carboplatin.

*Patient had primary mediastinal B-cell lymphoma.

†Total exceeds 100% as some patients received 2 lines of therapy.

Fifteen patients completed treatment according to protocol. Fourteen patients came off study because of disease progression or need for additional treatment before HDC-ASCT (12), AEs (1) and inadequate stem cell collection (1) (Figure 2, supplemental Figure 1).

Safety

No dose-limiting toxicity was noted at the 6 dose levels tested. CFZ 45 mg/m^2 was selected as the recommended dose for expansion. Median number of cycles of C-R-ICE was 3 (range, 2-3). Highest AE grade was 4 in 76% patients, 3 in 7% patients, and 2 in 17% patients. No patients experienced grade 5 toxicity. Majority of the grade 3 of 4 AEs were hematological (thrombocytopenia 72%; anemia 52%; neutropenia 31%; lymphopenia 3% and febrile neutropenia 10%) (Table 2). Nonhematological grade 3 of 4 AEs that occurred in >1 patient included hypokalemia (14%), hypophosphatemia (7%), and hypotension (7%). Among AEs of special interest pertaining to CFZ, 1 patient experienced grade 2 congestive heart failure (CHF), and 1 patient each had palpitations, sinus bradycardia, and sinus tachycardia. The patient with grade 2 CHF had hypertension at baseline. She was treated at dose level 1 of CFZ and developed CHF with cycle 1 in conjunction with gastrointestinal bleeding, anemia, and neutropenia. She recovered completely from the event with a preserved left ventricular ejection fraction and tolerated subsequent cycles well without any recurrence of CHF. There was no grade 3 of 4 cardiac AEs. The complete toxicity profile is presented in supplemental Table 2.

Antitumor activity

The best ORR was 66%, (95% CI, 46-82), and the CR rate was 48% for the entire cohort (n = 29) (Figure 3A). At the end of therapy, ORR was 62%, with a CR rate of 48%. Among the 14 patients treated with recommended dose for expansion of CFZ, both best and end of therapy ORR and CR rate were 71% and 50%, respectively (supplemental Table 1). There was a strong association between cell of origin and ORR, with most responses (17/18, 94%) occurring in the non-GCB phenotype (P = .001) (supplemental Table 2). ORR was 17 of 20 (85%) in patients with non-GCB DLBCL and 1 of 8 (13%) in GCB DLBCL, CR rate was 65% in non-GCB DLBCL and 13% in GCB DLBCL (Figure 3). One patient with PMBL (Primary Mediastinal Lymphoma) did not respond to therapy. Patients with primary refractory disease were less likely to respond to C-R-ICE with an ORR of 1 of 9 (11%) vs those with relapsed disease 17 of 20 (85%) (P = .001) (supplemental Figure 2A). Similarly, patients whose disease had relapsed within 12 months of diagnosis were less likely to achieve an ORR (3/11, 27%) compared with those whose disease had relapsed after 12 months from diagnosis (15/18, 83%) (P = .019) (supplemental Figure 2B). Of the 8 patients with GCB DLBCL, 5 had primary refractory disease (63%) of which 2 had progressive disease and 3 had stable disease as the best response. Of the 20 patients with non-GCB DLBCL, 3 (15%) had primary refractory disease of which 2 patients had progressive disease and 1 had PR as best response. Two additional patients had a disease relapse within 12 months of therapy and attained CR. There was no association between ORR and age, gender, Karnofsky Performance Scale at diagnosis, stage, bone marrow involvement, extranodal disease, IPI score or double hit status. Relationship between ORR and double expressor status could not be examined because of limited data available.

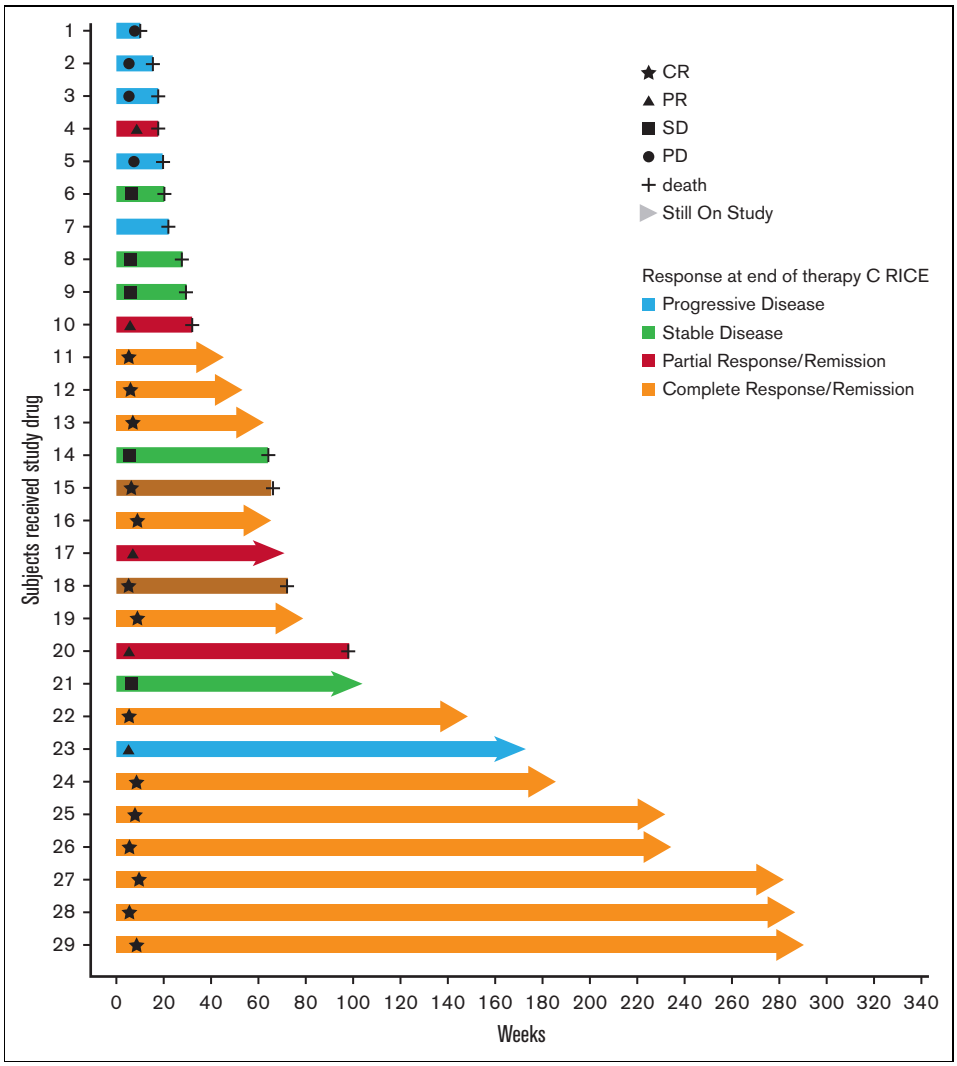

Figure 2. Patient disposition.

Fifteen (52%) patients underwent an autologous stem cell transplant. Reasons for deferring transplant included progressive disease (6), inadequate response (total 7; PR 3, stable disease 4), and inadequate stem cell collection (1). The median number of days of collection required was 3 (range, 2-9). Of 15 patients who underwent HDC-ASCT, 13 were in CR pretransplant, 1 in PR, and 1 was adjudicated as having stable disease. All 13 patients in CR pretransplant maintained their response at day +100. Of the 14 patients who attained CR at the end of C-R-ICE therapy, 3 patients experienced a disease relapse. Interestingly, 1 patient who attained CR but was unable to undergo HDC-ASCT because of inadequate collection, maintained his CR at last follow up. Subsequent therapy in the third-line setting included R-DHAC (5), gemcitabine and vinorelbine (1), rituximab and bendamustine (1), rituximab, polatuzumab-vedotin, and bendamustine (1), high-dose methotrexate (1), rituximab and lenalidomide (1), idelalisib (1), brentuximab-vedotin (1), CBL0137 on a clinical trial (1), pembrolizumab and dinaciclib on a clinical trial (1), and TRPH-222 on a clinical trial (1). Further lines of therapy included rituximab and lenalidomide (2), ibrutinib (2), rituximab, polatuzumab-vedotin, and bendamustine (2), pembrolizumab, R-DHAC (2), and tafasitamab-cxix and lenalidomide. Anti-CD19 chimeric antigen receptor T-cell (CAR-T) therapy was administered to 8 patients in the fourth line setting and beyond. At the time of data cut-off (March 2021), 14 patients had died, all of them because of disease progression.

Median follow-up was 40 months (range, 10.6-66.9 months). The median PFS was 15.2 months (5.1 months, not reached [NR]) with a 1-year PFS of 55% (95% CI, 0.36-0.71) (Figure 4A). Median OS was 22.6 months (range, 6.8; NR) with a 1-year OS of 66% (95% CI, 0.45-0.80) (Figure 4B). In the patients achieving CR at end of therapy, median PFS was 16.6 months (4.0 months, NR) and OS was 16.6 months (5.1 months, NR) with a 1-year PFS of 64% (95% CI, 0.34-0.83) and a 1-year OS of 71% (95% CI, 0.41-0.88). Achieving a CR as best response was significantly associated with longer PFS ($P < .0001$) and OS ($P < .0001$) (supplemental Figure 3). Similarly, achieving a CR at the end of therapy was significantly associated with longer PFS ($P < .0001$) and OS ($P = .0006$) (supplemental Figure 4). Patients with non-GCB DLBCL had a significantly longer PFS (NR vs 6.6 months; 1-year

Table 2. Toxicity profile

	Grade, n = 29, n (%)		
Adverse event	**Grade 3 any**	**Grade 4 any**	**Grade 3/4 total**
Anemia	14(48)	1(3)	15(52)
Febrile neutropenia	3(10)	0	3(10)
Leucopenia	0	5(17)	5(17)
Lymphopenia	0	1(3)	1(3)
Neutropenia	2(7)	7(24)	9(31)
Thrombocytopenia	2(7)	19(66)	21(72)
Abdominal pain	1(3)	0	1(3)
Gastric hemorrhage	1(3)	0	1(3)
Gastric obstruction	1(3)	0	1(3)
Device related infection	1(3)	0	1(3)
Lymph node abscess	1(3)	0	1(3)
Pneumonia	1(3)	0	1(3)
Sepsis	0(0.0)	1(3)	1(3)
Soft tissue infection	1(3)	0	1(3)
Dehydration	1(3)	0	1(3)
Hypocalcemia	0	1(3)	1(3)
Hyperglycemia	1(3)	0	1(3)
Hypokalemia	3(10)	1(3)	4(14)
Hyponatremia	1(3)	0	1(3)
Hypophosphatemia	1(3)	1(3)	2(7)
Back pain	1(3)	0	1(3)
Headache	1(3)	0	1(3)
Confusional state	1(3)	0	1(3)
Psychotic disorder	0	1(3)	1(3)
Dyspnea	1(3)	0	1(3)
Hypoxia	1(3)	0	1(3)
Pulmonary embolism	1(3)	0	1(3)
Hypertension	1(3)	0	1(3)
Hypotension	2(7)	0	2(7)
Adverse event	**Grade 1 (>10%)**	**Grade 2 (>10%)**	**Grade 1/2, total (>10%)**
Constipation	9(31)	5(17)	14(48)
Diarrhea	7(24)	5(17)	12(41)
Dry mouth	4(14)	0	4(14)
Dyspepsia	1(3)	4(14)	5(17)
Nausea	8(28)	12(41)	20(70)
Stomatitis	2(7)	2(7)	4(14)
Vomiting	4(14)	6(21)	10(35)
Asthenia	2(7)	2(7)	4(14)
Chills	5(17)	0	5(17)
Fatigue	10(35)	6(21)	16(55)
Edema	5(17)	1(3)	6(21)
Pyrexia	4(14)	0	4(14)
Infusion-related reaction	3(10)	4(14)	7(24)
Decreased appetite	2(7)	8(27.6)	10(35)
Muscular weakness	3(10)	2(7)	5(17)
Dizziness	4(14)	3(10)	7(24)

Table 2 (continued)

Adverse event	Grade, n = 29, n (%)		
	Grade 1 (>10%)	Grade 2 (>10%)	Grade 1/2, total (>10%)
Dysgeusia	6(21)	1(3)	7(24)
Headache	4(14)	3(10)	7(24)
Blurred vision	5(17)	0	5(17)
Peripheral sensory neuropathy	5(17)	0	5(17)
Tremor	2(7)	1(3)	3(10)
Insomnia	2(7)	2(7)	4(14)
Cough	7(24)	0	7 (24)
Dyspnea	3(10)	0	3(10)
Epistaxis	4(14)	0	4(14)
Alopecia	2(7)	11(38)	13(45)
Hypotension	0	3(10)	3(10)

PFS 65% vs 38%; $P = .0001$) and OS (NR vs 6.6 months; 1-year OS 80% vs 38%; $P < .0001$) (Figure 4C,D). Presence of primary refractory disease or relapse within 12 months of diagnosis was also associated with a significantly shorter PFS and OS, mirroring the response data (supplemental Figures 5 and 6).

Exploratory analysis

There was a statistically significant reduction in blood and peripheral blood mononuclear cell proteasomal activity after administration of CFZ on both days 1 and 2 of cycle 1, which was maintained on day 3 before administration of rituximab (supplemental Figures 7-11). The reduction was seen in all samples tested, irrespective of disease response. There was no association between degree of proteasomal inhibition and cell, origin, cytogenetics, or timing of relapse.

Discussion

In this phase 1 study of CFZ in combination with R-ICE regimen in R/R DLBCL, we show that addition of CFZ to R-ICE was well tolerated with no increase in toxicities beyond the expected AE profile of R-ICE. CFZ 45 mg/m^2 on days 1, 2, 8, and 9 was chosen to be the recommended dose for expansion. The ORR was 62% and CR rate was 48% with a median PFS of 15.2 months and a median OS of 22.6 months in the entire cohort. The non-GCB cohort seemed to benefit more from the C-R-ICE regimen compared with the GCB cohort with 65% of patients achieving a CR. Patients with primary refractory disease or those who relapsed within 12 months of diagnosis tended to do poorly with shorter survival. Patients with GCB DLBCL were more likely to have primary refractory disease in our study (63%) compared with those with non-GCB DLBCL (15%), which also accounts for the low response rates seen in GCB DLBCL.

Our results compare favorably to those reported with R-ICE in the CORAL study that reported an ORR of 63% and CR rate of 38% after induction chemotherapy. We show an improvement in CR rate of 10%, which is clinically meaningful considering that all our patients had received rituximab in the frontline setting as compared to only 62% in the CORAL study. Prior rituximab exposure was significantly associated with lower ORR (51%) in the CORAL study.[7] Similar to the CORAL study, only about 50% of patients in our study could proceed to HDC-ASCT, mainly because of inadequate disease response. One possible explanation for this may be that our cohort had a higher number of patients whose disease had relapsed within 12 months of diagnosis (38% vs 29% in the CORAL study), which is a well-established adverse prognostic factor.[14] Our data also compares favorably against other commonly used salvage chemotherapy regimens such as R-DHAP (ORR, 42% - 63%),[7,15,16] R-GDP (ORR, 44%),[15] and ofatumumab-DHAP (ORR, 38%).[16]

Our data confirmed the selective advantage of harnessing the nuclear factor kappa B pathway in non-GCB DLBCL with an ORR of 85% and CR rate of 65% in patients with non-GCB DLBCL. Other drugs have been used to harness the nuclear factor kappa pathway with similar results. Bortezomib, another proteasomal inhibitor, was combined with R-DA-EPOCH in 27 patients with R/R DLBCL with 83% ORR and 42% CRR in non-GCB DLBCL as compared with 13% ORR and 7% CRR in GCB DLBCL.[17] A phase 1 study evaluating ibrutinib in doses up to 840 mg daily in combination with R-ICE in R/R DLBCL showed impressive results with a CR rate of 89% in non-GCB DLBCL.[18] Combining lenalidomide with R-ICE led to a 60% ORR (9/15) after 2 cycles in patients with R/R DLBCL.[19] Although the early data looks quite promising, none of these strategies have found their way into clinical practice yet. Addition of CFZ to R-CHOP is also being investigated in non-GCB DLBCL in the frontline setting (NCT02073097).

What will be the relevance of our findings in the current era? Since the inception of this study, based on the ZUMA-7[20] and the TRANSFORM study,[21] anti-CD19 CAR-T therapy has been approved as a second-line option in patients with DLBCL that is refractory to or has relapsed within 12 months of first-line chemoimmunotherapy. Although CAR-T therapy represents a major advance in treatment of DLBCL, adequate disease control to allow for the time required for CAR-T cell processing remains a major challenge.[22] In fact, only 7% of patients in the ZUMA-7 study had non-GCB DLBCL, which may be secondary to the more aggressive clinical presentation of this phenotype and its higher prevalence in the older age group.[23] This is of particular interest in context of this study because C-R-ICE has preferential activity in non-GCB DLBCL. The most optimal bridging

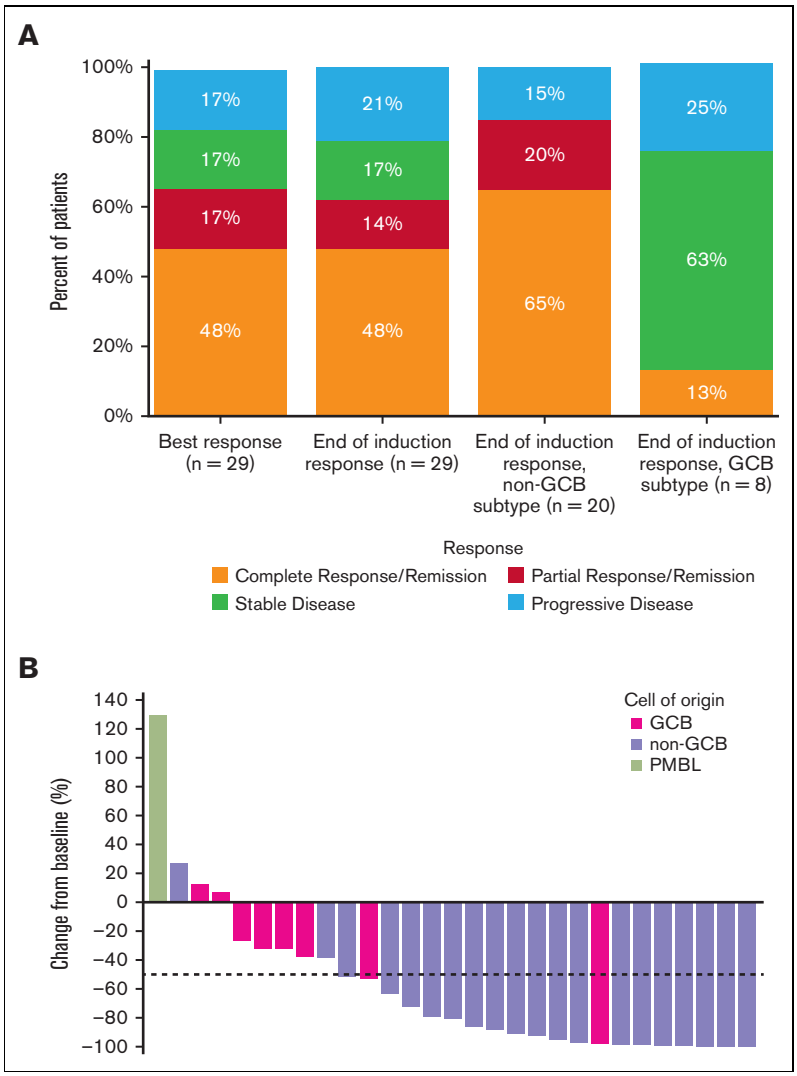

Figure 3. Response to therapy (n = 29). (A) Stacked bar chart showing best response, end of induction response and end of induction response according to cell of origin. One patient with primary mediastinal B-cell lymphoma had progressive disease. (B) Waterfall plot showing percentage of tumor shrinkage by cell of origin. PMBL, primary mediastinal B-cell lymphoma.

regimen pre–CAR-T therapy is yet to be determined. Because addition of CFZ clearly adds benefit to R-ICE without added toxicity, C-R-ICE could prove useful to achieve disease control pre–CAR-T therapy as it does not have deleterious effects on T cells such as that of bendamustine and does not utilize anti-CD19 strategies such as loncastuximab-tesirine or tafasitamab-cxix.[24-26] With the shifting landscape of DLBCL therapy, although HDC-ASCT might move to the third line setting in select patients, salvage regimens such as C-R-ICE would still be needed to determine chemosensitivity of the disease and attain a CR/PR before HDC-ASCT. Drawbacks of this approach include cumbersome IV dosing and multiple weekly visits for CFZ administration.

In summary, our study demonstrates that addition of CFZ to R-ICE is well tolerated and leads to high R/R, especially in patients with non-GCB DLBCL (85%). Patients with refractory DLBCL or those whose disease relapsed within 12 months of frontline therapy have significantly lower R/R to subsequent chemoimmunotherapy, and such patients should be considered for alternate therapies such as anti-CD19 CAR-T therapy.

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Authorship

Contribution: F.J.H.-I. and A.H. wrote the study; P.T., J.W., J.N., A.K., S.J.S., A.B., E.P., A.M., I.L., K.M., J.K., J.D., M.J., A.D., R.T., S.S., P.G. and F.J.H.-I. provided patient care; P.T., A.G., J.N., A.K., C.M., A.H., and F.J.H.-I were involved in translational studies, data collection/acquisition and/or analysis; P.T., A.G. and F.J.H.I were involved in clinical data interpretation; P.T., C.M., P.G., A.A.-G. and F.J.H.-I wrote the manuscript, and all authors approved the manuscript.

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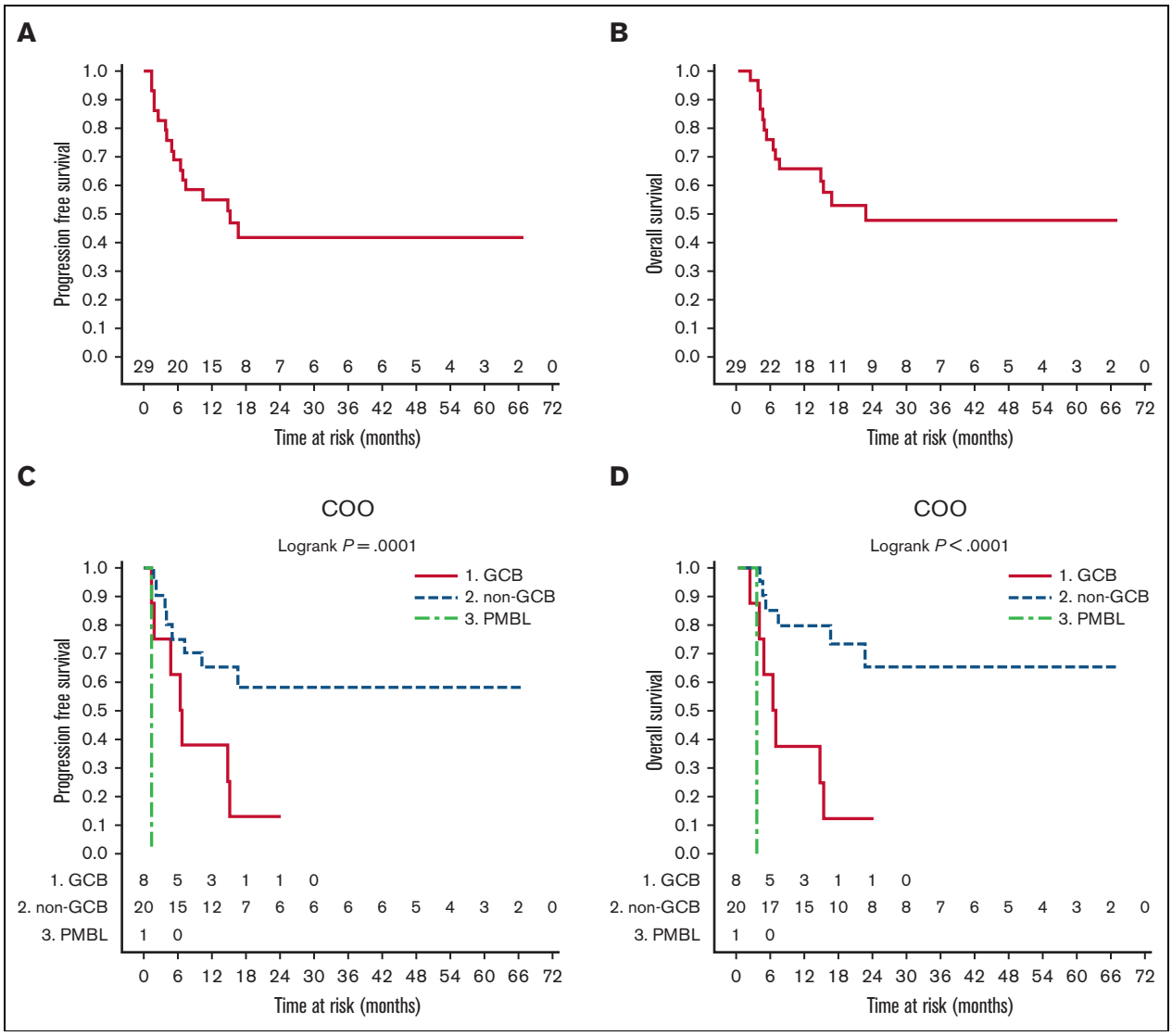

Figure 4. Kaplan-Meier survival curves. (A-D) PFS (A), and OS (B) curves of the entire cohort (n = 29). PFS (C), and OS (D) curves stratified by cell of origin. COO, cell of origin.

part of the Advisory Board for AstraZeneca, Daiichi Sankyo and Secura Bio. F.J.H.-I. is an advisory Board Member for Amgen, Karyopharm Therapeutics, Celgene Corporation, Kura Oncology, AbbVie Inc, Seattle Genetics Inc, Epizyme, Pharmacyclics, Gilead, Kite Pharma, Novartis, and MorphoSys. F.J.H.-I. is also a member of the Speaker Bureau of MorphoSys. The remaining authors declare no competing financial interests.

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References

1. Roschewski M, Staudt LM, Wilson WH. Diffuse large B-cell lymphoma-treatment approaches in the molecular era. *Nat Rev Clin Oncol*. 2014;11(1):12-23.
2. Project TIN-HsLPF. A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med*. 1993;329(14):987-994.
3. Sehn LH, Berry B, Chhanabhai M, et al. The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. *Blood*. 2007;109(5):1857-1861.

4. Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med*. 1995;333(23):1540-1545.
5. Gisselbrecht C, Van Den Neste E. How I manage patients with relapsed/refractory diffuse large B cell lymphoma. *Br J Haematol*. 2018;182(5):633-643.
6. Thieblemont C, Briere J, Mounier N, et al. The germinal center/activated B-cell subclassification has a prognostic impact for response to salvage therapy in relapsed/refractory diffuse large B-cell lymphoma: a bio-CORAL study. *J Clin Oncol*. 2011;29(31):4079-4087.
7. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol*. 2010;28(27):4184-4190.
8. Sauter CS, Matasar MJ, Meikle J, et al. Prognostic value of FDG-PET prior to autologous stem cell transplantation for relapsed and refractory diffuse large B-cell lymphoma. *Blood*. 2015;125(16):2579-2581.
9. Olejniczak SH, Hernandez-Ilizaliturri FJ, Clements JL, Czuczman MS. Acquired resistance to rituximab is associated with chemotherapy resistance resulting from decreased Bax and Bak expression. *Clin Cancer Res*. 2008;14(5):1550-1560.
10. Olejniczak SH, Blickwedehl J, Belicha-Villanueva A, et al. Distinct molecular mechanisms responsible for bortezomib-induced death of therapy-resistant versus -sensitive B-NHL cells. *Blood*. 2010;116(25):5605-5614.
11. Yee AJ. The role of carfilzomib in relapsed/refractory multiple myeloma. *Ther Adv Hematol*. 2021;12:1-13:20406207211019612.
12. Gu JJ, Hernandez-Ilizaliturri FJ, Kaufman GP, et al. The novel proteasome inhibitor carfilzomib induces cell cycle arrest, apoptosis and potentiates the anti-tumour activity of chemotherapy in rituximab-resistant lymphoma. *Br J Haematol*. 2013;162(5):657-669.
13. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. *J Clin Oncol*. 2007;25(5):579-586.
14. Liu Y, Barta SK. Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. *Am J Hematol*. 2019;94(5):604-616.
15. Crump M, Kuruvilla J, Couban S, et al. Randomized comparison of gemcitabine, dexamethasone, and cisplatin versus dexamethasone, cytarabine, and cisplatin chemotherapy before autologous stem-cell transplantation for relapsed and refractory aggressive lymphomas: NCIC-CTG LY.12. *J Clin Oncol*. 2014;32(31):3490-3496.
16. van Imhoff GW, McMillan A, Matasar MJ, et al. Ofatumumab versus rituximab salvage chemoimmunotherapy in relapsed or refractory diffuse large B-Cell lymphoma: The ORCHARRD study. *J Clin Oncol*. 2017;35(5):544-551.
17. Dunleavy K, Pittaluga S, Czuczman MS, et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. *Blood*. 2009;113(24):6069-6076.
18. Sauter CS, Matasar MJ, Schoder H, et al. A phase 1 study of ibrutinib in combination with R-ICE in patients with relapsed or primary refractory DLBCL. *Blood*. 2018;131(16):1805-1808.
19. Feldman T, Mato AR, Chow KF, et al. Addition of lenalidomide to rituximab, ifosfamide, carboplatin, etoposide (RICER) in first-relapse/primary refractory diffuse large B-cell lymphoma. *Br J Haematol*. 2014;166(1):77-83.
20. Locke FL, Miklos DB, Jacobson CA, et al. Axicabtagene Ciloleucel as Second-Line Therapy for Large B-Cell Lymphoma. *N Engl J Med*. 2021;386(7):640-654.
21. Kamdar M, Solomon SR, Arnason JE, et al. Lisocabtagene maraleucel (liso-cel), a CD19-directed chimeric antigen receptor (CAR) T cell therapy, versus standard of care (SOC) with salvage chemotherapy (CT) followed by autologous stem cell transplantation (ASCT) as second-line (2L) treatment in patients (Pts) with relapsed or refractory (R/R) large B-cell lymphoma (LBCL): results from the randomized phase 3 Transform study. *Blood*. 2021;138(Supplement 1):91.
22. Bishop MR, Dickinson M, Purtill D, et al. Second-line tisagenlecleucel or standard care in aggressive B-Cell lymphoma. *N Engl J Med*. 2021;386(7):629-639.
23. Di M, Huntington SF, Olszewski AJ. Challenges and opportunities in the management of diffuse large B-cell lymphoma in older patients. *Oncol*. 2021;26(2):120-132.
24. Nizamuddin I, David KA, Cohen JB, et al. Practice patterns pre-CART for aggressive B-cell lymphomas: patient selection and real-world salvage and bridging practices. *Blood*. 2021;138(suppl 1):532.
25. Sehn LH, Herrera AF, Flowers CR, et al. Polatuzumab vedotin in relapsed or refractory diffuse large B-Cell lymphoma. *J Clin Oncol*. 2020;38(2):155-165.
26. Tilly H, Morschhauser F, Sehn LH, et al. Polatuzumab vedotin in previously untreated diffuse large B-Cell lymphoma. *N Engl J Med*. 2021;386(4):351-363.