Acalabrutinib for treatment of diffuse large B-cell lymphoma: results from a phase Ib study

Two major molecular subtypes of diffuse large B-cell lymphoma (DLBCL), germinal center B-cell (GCB) and activated B-cell (ABC), have been defined from gene expression studies and are recognized under the World Health Organization 2016 classification.[1,2] Genetic abnormalities associated with ABC DLBCL result in chronic active B-cell receptor (BCR) signaling and NF-κB pathway activation.[3] Bruton tyrosine kinase (BTK) plays a key role in the BCR signaling pathway. Covalent irreversible BTK inhibitors have recently been studied for treatment of B-cell malignancies, including relapsed/refractory (R/R) DLBCL. The response rate in a study of ibrutinib was higher in patients with ABC DLBCL than with GCB DLBCL (37% [14 of 38] *vs.* 5% [one of 20]).[4] Similarly, for tirabrutinib, the response rate in non-GCB DLBCL was 35% (11 of 31); neither of the two patients with GCB subtype disease responded.[5] While median duration of response to ibrutinib was 4.83 months, four patients had durable complete responses (CR) >24 months.[4] Acalabrutinib is approved for the treatment of patients with the B-cell malignancies of chronic lymphocytic leukemia (CLL) and R/R mantle cell lymphoma (MCL).[6] It is a selective, covalent BTK inhibitor, possibly having fewer off-target biological effects and a better clinical safety profile than ibrutinib.[7] The objective of this multi-center, open-label phase Ib study was to investigate the safety, pharmacokinetics, pharmacodynamics, and efficacy of acalabrutinib monotherapy in patients with R/R non-GCB DLBCL (clinicaltrials gov. Identifier: NCT02112526). We found that the safety profile of acal-

Table 1. Incidence of treatment-emergent and treatment-related adverse events in ≥2 patients (n=21).

Preferred term[a], n (%)	Treatment-Emergent Adverse Events		Treatment-Related Adverse Events	
	All Grades	Grade ≥3	All Grades	Grade ≥3
Diarrhea	9 (42.9)	1 (4.8)	4 (19.0)	1 (4.8)
Fatigue	9 (42.9)	2 (9.5)	4 (19.0)	–
Anemia	6 (28.6)	5 (23.8)	3 (14.3)	3 (14.3)
Cough	6 (28.6)	–	3 (14.3)	–
Dizziness	6 (28.6)	1 (4.8)	5 (23.8)	1 (4.8)
Headache	5 (23.8)	–	2 (9.5)	–
Nausea	5 (23.8)	–	2 (9.5)	–
Constipation	4 (19.0)	–	–	–
Decreased appetite	4 (19.0)	1 (4.8)	–	–
Dyspnea	4 (19.0)	1 (4.8)	–	–
Peripheral edema	4 (19.0)	–	2 (9.5)	–
Arthralgia	3 (14.3)	1 (4.8)	–	–
Dehydration	3 (14.3)	1 (4.8)	–	–
Hypokalemia	3 (14.3)	–	–	–
Pyrexia	3 (14.3)	1 (4.8)	3 (14.3)	1 (4.8)
Abdominal pain	2 (9.5)	2 (9.5)	–	–
Back pain	2 (9.5)	–	–	–
Contusion	2 (9.5)	–	–	–
Ecchymosis	2 (9.5)	–	–	–
Gait disturbance	2 (9.5)	1 (4.8)	–	–
Hypomagnesemia	2 (9.5)	–	–	–
Insomnia	2 (9.5)	–	–	–
Musculoskeletal pain	2 (9.5)	–	–	–
Nasopharyngitis	2 (9.5)	–	–	–
Neck pain	2 (9.5)	–	–	–
Oral candidiasis	2 (9.5)	–	–	–
Pain in extremity	2 (9.5)	–	–	–
Petechiae	2 (9.5)	–	2 (9.5)	–
Pleural effusion	2 (9.5)	–	–	–
Rales	2 (9.5)	–	–	–
Rash	2 (9.5)	–	2 (9.5)	–
Respiratory failure	2 (9.5)	2 (9.5)		
Sinusitis	2 (9.5)	–	–	–
Vomiting	2 (9.5)	–	–	–

[a]National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

abrutinib in R/R DLBCL patients was consistent with previous studies of acalabrutinib. In addition, the observed efficacy, pharmacokinetic, and pharmacodynamic activity in this study supports further evaluation of acalabrutinib-based combinations in DLBCL.

Non-GCB DLBCL was identified per local institution immunohistochemistry using the Hans algorithm.[8] A subset of patients with available tissue had central cell-of-origin testing performed by RNA profiling (NanoString Lymphoma Subtyping Test). Patients received acalabrutinib 100 mg twice daily in repeated 28-day cycles until disease progression or unacceptable drug-related toxicity.

The primary endpoint was safety, measured by the incidence of adverse events (AE) from screening up to 30 days after the last acalabrutinib dose. Secondary endpoints included acalabrutinib pharmacokinetics, pharmacodynamics (BTK occupancy), and efficacy. Plasma pharmacokinetic parameters were determined from plasma concentrations of acalabrutinib using non-compartmental analysis. BTK occupancy was measured with enzyme-linked immunsorbent assay using a biotin-tagged analogue probe.[7] Efficacy was measured according to investigator-assessed objective response rate (ORR: CR + partial response [PR]) based on the Lugano criteria,[9] duration of response, and progression-free survival (PFS).
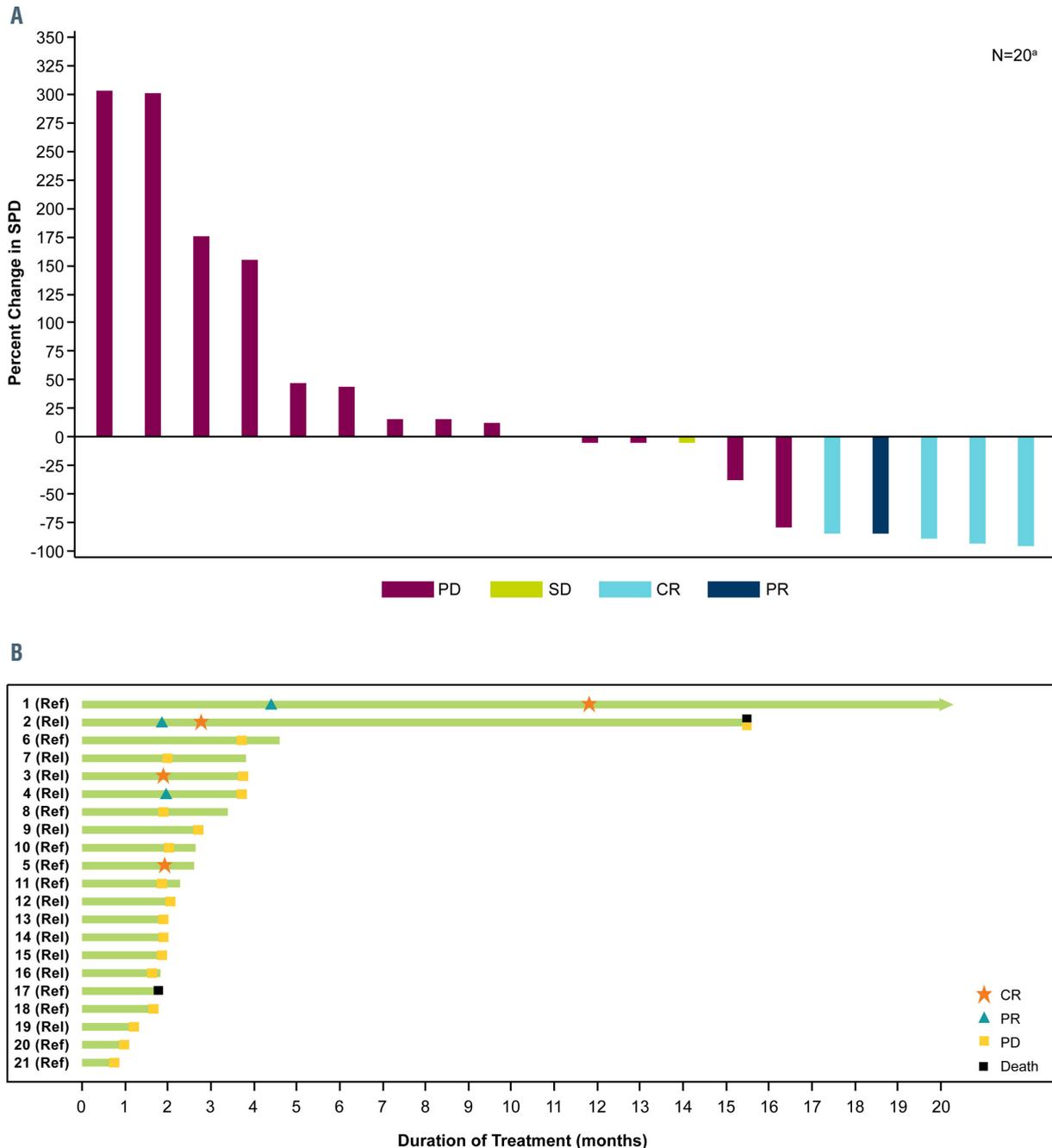
Twenty-one patients were enrolled; demographics and

Figure 1. Patient-level responses. (A) Best response and maximum change in tumor burden by patient. (B) Duration of treatment with best response by patient. Arrow in panel (B) indicates the patient is continuing treatment with acalabrutinib. [a]Best response for one patient was unknown and thus not included in this graph. CR: complete response; ORR: overall response rate; PD: progressive disease; PR: partial response; Ref: refractory; Rel: relapsed; SD: stable disease.
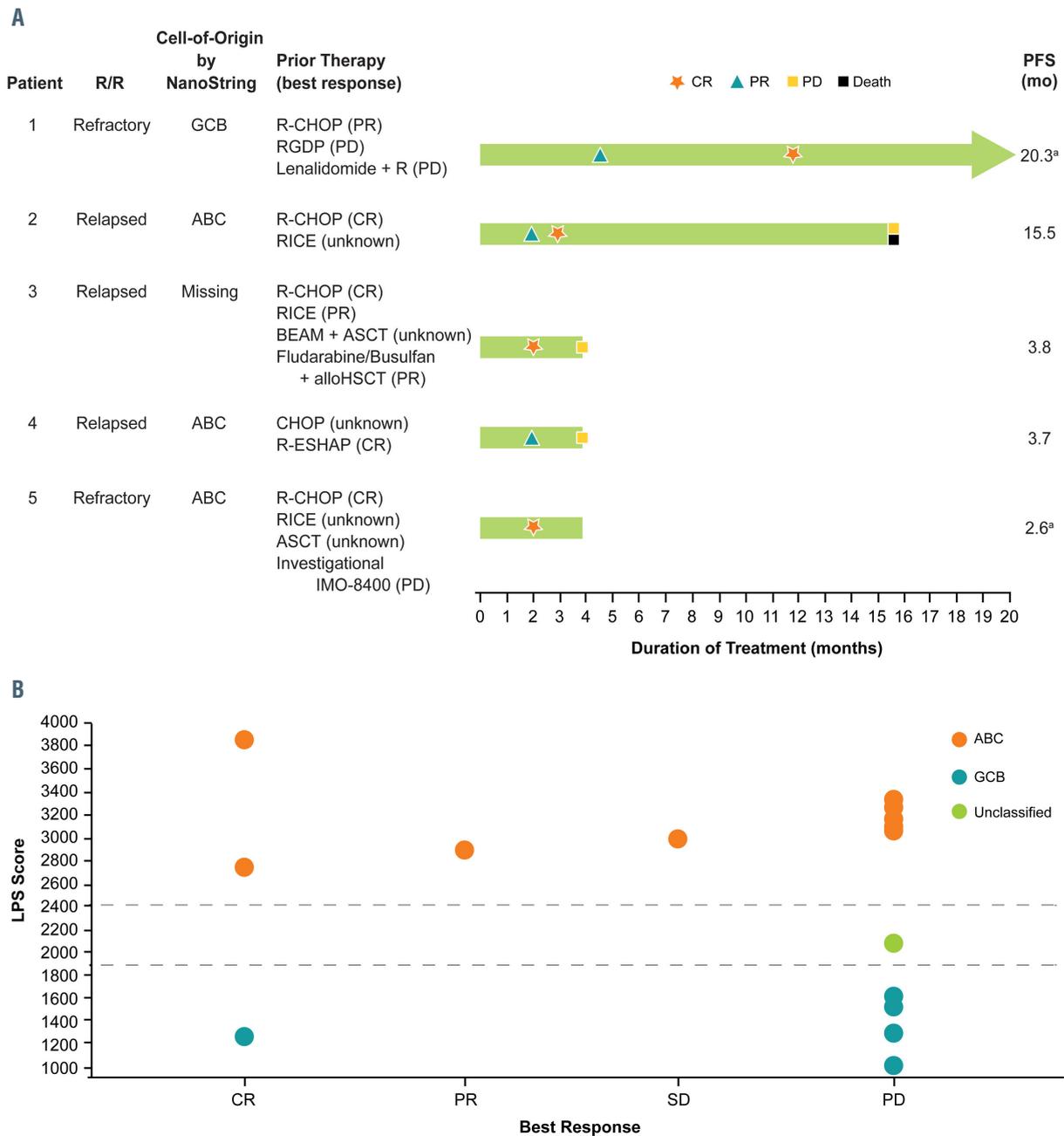
A

B

Figure 2. Cell of origin by NanoString. (A) Responders including duration of response. (B) Best overall response in the 15 patients who had evaluable samples available for NanoString subtyping. All enrolled patients (n=21) had local non–germinal center B-cell (GCB) immunohistochemistry cell-of-origin testing. Archival tumor samples were submitted for central gene sequencing analysis (n=15) using the Lymphoma Subtyping Test (LST) by NanoString platform (Covance Genomics Labs). [a]Censored. ASCT: autologous stem cell transplantation; BEAM: BiCNU (carmustine), etoposide, Ara-C (cytarabine), and melphalan; CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisolone; CR: complete response; GCB: germinal center B-cell; HSCT: hematopoietic stem cell transplantation; LPS: linear predictor score; PD: progressive disease; PFS: progression-free survival; PR: partial response; R: rituximab; R/R: relapsed/refractory; R-CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisolone + rituximab; R-ESHAP: rituximab, etoposide, solu-medrone, high-dose cytarabine, and cisplatin; RGDB: cisplatin, dexamethasone, gemcitabine and rituximab; RICE: rituximab, ifosfamide, carboplatin, and etoposide; SD: stable disease.

baseline characteristics are shown in the *Online Supplementary Table S1*. Median age was 64 years (range, 32–84), 48% were male, 71% were white, 67% had an ECOG PS of 1, 57% had Ann Arbor stage IV disease at baseline, and 24% had had prior autologous stem cell transplantation. Median number of prior systemic regimens was 3 (range, 1–5), and 81% had received prior R-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone + rituximab). As of the data cutoff date of 30 October 2017, 95% (20 of 21) discontinued the study, 81% (17 of 21) due to disease progression, 9.5% (two of 21) due to AE listed as grade 5 (one respiratory failure with grade 4 pneumonia and lower respiratory *Staphylococcus aureus* infection not related to treatment, and one with leptomeningeal involvement of DLBCL), and 4.8% (one of 21) to pursue an alternative cancer therapy (stem cell transplant) while in CR. There were eight deaths, none considered related to acalabrutinib. One

patient remained on acalabrutinib at the time of data cut-off; this patient has maintained CR since December 2016 through the last follow-up assessment (August 2020).

Median duration of exposure to acalabrutinib was 2.3 months (range, 0.5–22.5), with a median relative dose intensity of 98.1% (range, 6.3–100.0%). Two patients had a dose reduction, one due to grade 2 orthostatic hypotension, and the other due to prescription of a strong CYP3A inhibitor. Three (14.3%) patients had ≥1 doses withheld for ≥7 days, including two due to AE (both grade 3 abdominal pain considered not related to the study drug).

Treatment-emergent AE occurred in 95% of patients, most commonly diarrhea (43%) and fatigue (43%); 29% experienced grade 3/4 AE (Table 1). Treatment-related AE occurred in 16 (76%) patients; five (24%) experienced grade 3/4 treatment-related AE; two patients developed grade 3 serious AE that were considered treatment-related (pyrexia in one patient, and positive influenza B virus test in the other patient).

Regarding AE of clinical interest, no atrial fibrillation, hypertension, tumor lysis syndrome, or grade ≥3 bleeding were reported; any-grade AE of special interest included hemorrhage in eight (38%) and infections in 11 (52%) patients, with three patients (14%) having grade ≥3 infections. Grade ≥3 infections included one patient with grade 4 pneumonia (lower respiratory culture identified *Staphylococcus aureus*), one patient with grade 3 rhinovirus infection, staphylococcal infection, staphylococcal urinary tract infection, and grade 5 septic shock, and one patient with grade 3 viral infection. Several factors may have contributed to the lack of atrial fibrillation and hypertension reported in this study, beyond the acalabrutinib selectivity for BTK, including comorbid health conditions, small population sample, and short follow-up/drug exposure duration.

The ORR was 24% (n=5 of 21), with four patients achieving a CR and one experiencing a PR; one patient had stable disease (SD) (Figure 1A and 1B). Median duration of response for the five responders was 7.8 months (95% Confidence Interval [CI]: 1.8-not reached). NanoString subtyping was available for 15 patients and revealed nine ABC, five GCB, and one unclassified subtype (Figure 2). ORR in patients with ABC subtype by NanoString was 33% (three of nine), with 22% (two of nine) achieving CR. Among patients with GCB subtype by gene expression, one achieved CR; none achieved PR. Duration of response was substantially longer in two of the patients with CR than in the majority of patients (Figure 1B). One patient with ABC subtype disease experienced PD after 13.7 months; the other, who remained on treatment at the time of data cutoff (15.9 months) had GCB subtype disease, in a background rich in T cells and histiocytes (Figure 2A). This latter patient had had a PR to prior R-CHOP and experienced PD on regimens of rituximab/gemcitabine/dexamethasone/cisplatin and of lenalidomide+rituximab.

Median PFS and overall survival based on Kaplan-Meier analysis were 1.9 months (95% CI: 1.8-2.7) and 15.5 months (95% CI: 4.0-not reached), respectively (*Online Supplementary Figures S1A* and *B*). For the 11 patients who received subsequent lines of therapy, median time to next treatment was 4.0 months (95% CI: 3.0-7.5).

The pharmacokinetics of acalabrutinib were characterized by rapid absorption and elimination, indicating low potential for accumulation. These results were consistent with previous studies in patients with CLL and MCL.[7,10]

Among 14 patients for whom pharmacokinetic data were available, comparable steady-state mean maximum concentration (C_{max}) and area under the concentration time curve (AUC_{last}) were observed between responders (n=5) and non-responders (n=9) (*Online Supplementary Figure S2*). BTK receptor occupancy data were available for five patients with evaluable predose and postdose samples, including three responders and two non-responders. Consistent with previous results in CLL and MCL,[7,10] median steady-state BTK target occupancy was 97% to 99% throughout the dosing interval, and was >90% for all patients at all time points (irrespective of clinical responses), indicating complete target coverage by acalabrutinib (*data not shown*).

Acalabrutinib monotherapy demonstrated activity in R/R DLBCL patients, some with durable responses. The ORR of 24%, with a rate of 33% in ABC subtype, was overall consistent with that reported for ibrutinib and tirabrutinib.[4,5] Nevertheless, one patient of confirmed GCB subtype who had documented primary resistance to R-CHOP chemotherapy entered a durable CR, which persists over 4 years since commencing therapy. Comparable exceptional responders have been observed with ibrutinib, but these cases were of ABC subtype.

Overall, BTK inhibitors appear to be moderately effective in ABC DLBCL,[4] possibly due to the necessity for maintained BCR signaling; BTK inhibition thus results in apoptosis, as observed *in vitro* in some ABC cell lines.[11] However, in most patients with ABC DLBCL, responses, if present, are usually short-lived[4]; mechanisms behind resistance or short duration of response in DLBCL are not known, precluding prospective identification of patients who would benefit most from this therapy. This problem is highlighted by two observations. Firstly, a study of tirabrutinib in primary central nervous system DLBCL found it to be effective against DLBCL with *CARD11* mutations in the coiled-coil domain, suggesting that a more precise molecular understanding of the B-cell receptor pathway will help better guide patient selection for this treatment.[12,13] Secondly, the best responder in our study was of GCB subtype (though rich in T cells and histiocytes; subtyping determined via NanoString Lymphoma Subtyping Test), suggesting that some patients with this subtype can have durable responses to BTK inhibition. This durable response in GCB disease perhaps reflects the fact that there are multiple genomic subtypes across ABC and GCB DLBCL, each with different risk profiles and expected responsiveness to BTK inhibitors.[14-16] Given that inclusion criteria required immunohistochemically confirmed non-GCB DLBCL, the presence of patients with GCB within this cohort highlights the variable concordance between immunohistochemistry algorithms and gene-expression profiling and the potential benefits of genomic profiling in defining treatment subgroups.[17] Furthermore, genomic profiling beyond gene expression analysis may also represent a better means of selecting patients who may respond to different treatment modalities compared with using cell-of-origin classification. The incidental inclusion of patients with GCB DLBCL may be a limitation of this study given the known sensitivity of non-GCB DLBCL to BTK-dependent B-cell receptor signaling inhibition and the reported preferential activity of BTK inhibitors in the non-GCB DLBCL subtype.[4]

Macrophage impairment that has been observed with ibrutinib[18,19] has not been observed with acalabrutinib; in addition, the comparable efficacy in ABC DLBCL to other BTK inhibitors[4,5] and the acceptable toxicity profile sug-

gest that acalabrutinib might be a preferable option for BTK inhibitor–based combination therapy. The safety profile of acalabrutinib in R/R DLBCL patients was consistent with previous studies; the most common AE were mainly grades 1 or 2, and did not lead to treatment discontinuation. The observed activity and favorable safety profile support evaluation of acalabrutinib-based combinations in DLBCL. Several clinical trials evaluating the combination of acalabrutinib with chemotherapy in patients with DLBCL are underway, including a phase I/II trial (clinicaltrials.gov Identifier: NCT03571308) that is investigating acalabrutinib in combination with R-CHOP in patients with untreated DLBCL expressing CD20, a phase II trial (clinicaltrials.gov Identifier: NCT04002947) that is studying acalabrutinib in combination with R-CHOP or DA-EPOCH in patients with untreated DLBCL, and a phase III trial that is evaluating the addition of acalabrutinib to R-CHOP in patients with untreated non-GCB DLBCL (clinicaltrials.gov Identifier: NCT04529772).

Paolo Strati,[1] Sven de Vos,[2] Jia Ruan,[3] Kami J. Maddocks,[4] Christopher R. Flowers,[1,5] Simon Rule,[6] Priti Patel,[7] Yan Xu,[7] Helen Wei,[7] Melanie M. Frigault,[7] Roser Calvo[8] and Martin J.S. Dyer[9]

[1]Department of Lymphoma and Myeloma, University of Texas MD Anderson Cancer Center, Houston, TX, USA; [2]Division of Hematology and Oncology, Department of Medicine, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA, USA; [3]Division of Hematology and Medical Oncology, Meyer Cancer Center, Weill Cornell Medicine, New York, NY, USA; [4]Division of Hematology, The James Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA; [5]Winship Cancer Institute of Emory University, Atlanta, GA, USA; [6]Department of Hematology, Plymouth University Medical School, Plymouth, UK; [7]AstraZeneca, South San Francisco, CA, USA; [8]AstraZeneca, Gaithersburg, MD, USA and [9]Ernest and Helen Scott Hematological Research Institute, University of Leicester, Leicester, UK

Correspondence:

MARTIN J.S. DYER - mjsd1@le.ac.uk

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