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Journal

Clinical Cancer Research, 29(17)

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Publication Date

2023-09-01

DOI

10.1158/1078-0432.CCR-22-2483

Peer reviewed

Phase I Study of Acalabrutinib Plus Danvatirsen (AZD9150) in Relapsed/Refractory Diffuse Large B-Cell Lymphoma Including Circulating Tumor DNA Biomarker Assessment

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ABSTRACT

Purpose: Novel targeted and immunotherapies have improved outcomes in relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL), but toxicities limit widespread use. The selective Bruton tyrosine kinase (BTK) inhibitor acalabrutinib has activity in patients with R/R DLBCL but durable responses are uncommon. STAT3 inhibition has demonstrated clinical activity in DLBCL.

Patients and Methods: Final results of the phase I study of acalabrutinib plus STAT3 inhibitor (danvatirsen; AZD9150) in patients with R/R DLBCL are reported. Danvatirsen 200 mg intravenous infusion [Days 1, 3, 5 (Cycle 1); weekly infusions starting Day 8, Cycle 1] was administered in combination with oral acalabrutinib 100 mg twice daily until progressive disease (PD) or unacceptable toxicity. Primary endpoints were safety and tolerability. Secondary endpoints included efficacy, pharmacokinetics, and immunogenicity.

Results: Seventeen patients received combination treatment. One dose-limiting toxicity (Grade 3 liver transaminase) occurred in 1 patient. The most common reason for treatment discontinuation was PD (65%). In evaluable patients ($n = 17$), objective response rate was 24%; median duration of response was 1.9 months. All responders with available DLBCL cell-of-origin data were either activated B-cell or nongerminal center B-cell like subtype. Genetic subtype did not correlate with response. Baseline and longitudinal plasma cell-free DNA (cfDNA) concentrations were mostly higher in nonresponding patients. cfDNA changes were generally concordant with imaging. Pretreatment circulating B-cell levels were higher in responders versus nonresponders.

Conclusions: Targeting both STAT3 and BTK in combination is safe and tolerable but efficacy is limited in R/R DLBCL. Results support evaluation of circulating tumor DNA as a biomarker for clinical response.

Introduction

Patients with relapsed/refractory (R/R) diffuse large B-cell lymphoma (DLBCL) after prior autologous stem cell transplant (ASCT) or chimeric antigen receptor T-cell (CAR-T) therapy have poor outcomes with limited treatment options (1). Development of rational drug combinations targeting complementary oncogenic signaling pathways in R/R DLBCL may improve outcomes. One rational target is Bruton tyrosine kinase (BTK), a component of the B-cell receptor signaling pathway that plays a key role in B-cell lymphomas, including activated B-cell (ABC) DLBCL (2). BTK inhibitors have demonstrated safety and activity in subsets of patients with R/R DLBCL, including those with nongerminal B-cell and ABC DLBCL, but remission durations are short (3, 4).

High levels of STAT3 expression and activation have been preferentially detected in ABC DLBCL cells (5), and STAT3 inhibition has suppressed the growth of DLBCL xenografts in preclinical models (6). Danvatirsen (AZD9150; ISIS 481464) is a phosphorothioate-modified chimeric generation 2.5 antisense oligonucleotide (16-mer ASO) designed to target and downregulate human STAT3 mRNA expression. Danvatirsen comprises a 10-base central oligodeoxynucleotide core that supports RNase H1-mediated cleavage of STAT3 mRNA, flanked by three constrained ethyl (cEt)-modified nucleosides on both the 5′ and 3′ ends (7). A phase Ib study demonstrated the safety and tolerability of danvatirsen in DLBCL with evidence of clinical activity including two complete responses (CR) and two partial responses (PR) in 27 patients (8).

PRISM is a phase I master protocol study designed to evaluate multiple targeted therapies alone or in combination in patients with R/R aggressive non-Hodgkin lymphoma (NHL). Here, we report results from Arm 1 of the PRISM study that evaluated combination therapy with the highly selective, covalent BTK

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Clin Cancer Res 2023;29:3301–12

doi: 10.1158/1078-0432.CCR-22-2483

Translational Relevance

Diffuse large B-cell lymphoma (DLBCL) is a genetically and clinically heterogenous disease with varied outcomes following front-line treatment. Prognosis remains poor in patients with relapsed/refractory (R/R) DLBCL, with limited treatment options after prior autologous stem cell transplant or chimeric antigen receptor T-cell therapy. Rational drug combinations targeting underlying molecular mechanisms in R/R DLBCL may improve outcomes. We report the results from one arm of the phase I master protocol PRISM study that evaluated combination treatment with the Bruton tyrosine kinase (BTK) inhibitor acalabrutinib and the anti-STAT3 oligonucleotide danvatirsen (AZD9150) in 17 patients with R/R DLBCL. Overall response rate was 24%. Patterns of mutational frequencies differed on the basis of treatment response, with decreased levels of cell-free DNA concentrations and allele frequency of mutations in plasma identified in treatment responders. Although our findings support the rationale for simultaneous targeting of STAT3 and BTK, limited clinical benefit was seen in this small R/R DLBCL population.

inhibitor acalabrutinib and the anti-STAT3 allele-specific oligonucleotide danvatirsen in patients with R/R DLBCL.

Patients and Methods

Study design and treatment

PRISM was an exploratory phase, open-label, nonrandomized, multicenter, phase I platform study (NCT03527147) with four treatment arms designed to assess the safety, efficacy, pharmacokinetics (PK), and pharmacodynamics of acalabrutinib in combination with either danvatirsen (STAT3 inhibitor), AZD6738 [ataxia telangiectasia and Rad3-related kinase (ATR) inhibitor], Hu5F9-G4 plus rituximab (anti-CD47 antibody plus anti-CD20 mAb), or AZD5153 [bromodomain-containing protein 4 (BRD4) inhibitor] in patients with R/R DLBCL (Supplementary Fig. S1). Module 1 was conducted at 10 sites in the United States and two sites in the United Kingdom. The data presented herein correspond to the acalabrutinib plus danvatirsen arm. Patients received acalabrutinib 100 mg orally twice daily starting on Cycle 1, Day 1, and continuing until disease progression, unacceptable toxicity, or treatment discontinuation due to other reasons. Danvatirsen 200 mg was administered via 1-hour intravenous infusion on Days 1, 3, and 5 of Cycle 1, followed by weekly infusions starting on Cycle 1, Day 8 until disease progression, unacceptable toxicity, or treatment discontinuation for other reasons. No dose escalation was conducted. All patients were followed for survival until death, loss to follow-up, the decision to stop the trial, or withdrawal of consent.

The study complied with the principles of the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. The study protocol was approved by the local ethics committee/institutional review board. All study participants provided written informed consent.

Study participants

Eligible patients were ages ≥18 years with a diagnosis of R/R DLBCL according to WHO criteria, Eastern Cooperative Oncology Group (ECOG) performance status ≤2, and adequate hepatic and renal function. Patients must have previously received ≥1 line of prior chemoimmunotherapy [e.g., rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP) or equivalent regimen and high-dose therapy with stem-cell rescue] or been ineligible for high-dose chemotherapy with stem-cell rescue and/or CAR-T therapy. Key exclusion criteria are presented in Supplementary Table S1.

Study objectives and assessments

Primary endpoint: safety

The primary objective was to evaluate the safety of acalabrutinib in combination with danvatirsen for the treatment of R/R DLBCL. Safety was assessed by adverse events (AE), abnormalities of laboratory tests, serious AEs, and AEs leading to treatment discontinuation. Dose-limiting toxicities (DLT) were assessed and included any Grade 5 toxicity; any uncontrolled Grade ≥3 nonhematologic toxicity (except nausea); hematologic toxicities [Grade 4 neutrophil count decrease >5 days, febrile neutropenia (Grade 3 requiring hospitalization or Grade 4), platelet count decrease (Grade 3 with Grade ≥3 bleeding or Grade 4), or unexplained Grade 4 anemia]; Grade ≥3 liver transaminase elevation; tumor lysis syndrome (TLS) without resolution of metabolic abnormalities within 72 hours despite protocol-required management; clinical TLS despite protocol-specified management; and dose delay due to drug-related toxicity (>21 days for acalabrutinib; >14 days for danvatirsen).

Secondary endpoints: clinical activity, pharmacokinetics, and immunogenicity

Clinical activity was a secondary objective; assessments included overall response rate (ORR) based on International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017; ref. 9); duration of response (DOR); progression-free survival (PFS); and overall survival (OS). Tumor assessments by PET or CT scan were conducted at screening and every 8 weeks. PFS was defined as the time from first dose date to documented objective disease progression, or death from any cause, whichever occurred first. OS was defined as the time from first dose to the date of death from any cause.

Assessing the PK of acalabrutinib, ACP-5862 (major active metabolite of acalabrutinib), and danvatirsen was a secondary objective. Pharmacokinetic sampling for measuring acalabrutinib, ACP-5862, and danvatirsen in plasma was conducted on Cycle 1, Day 8, predose and 1, 4, and 6 hours postdose. Pharmacokinetic sampling for measuring danvatirsen in plasma also was done predose and at the end of infusion on Day 1 of every odd cycle starting with Cycle 3.

Assessing immunogenicity to danvatirsen was a secondary objective. Blood samples for determination of potential anti-danvatirsen antibody formation were collected predose from all subjects treated with danvatirsen on Days 1 and 8 of Cycle 1 and then predose on Day 1 of every odd cycle of danvatirsen beginning with Cycle 3. Presence of antidrug antibodies was assessed using a validated enzyme-linked immunosorbent assay (ELISA).

Exploratory assessments

Immunophenotyping. Immunophenotyping was conducted (Clinical Laboratory Services by Q^2 Solutions) by flow cytometry analyses of peripheral blood mononuclear cells. Frequency and absolute numbers of immune cell populations, including B cells, T cells, natural killer (NK) cells, and monocytes, were analyzed using peripheral blood collected predose (Cycle 1, Day 1) and longitudinally using CD45 (BD Biosciences, Catalog No. 560777, RRID:AB_1937324), CD3 (BD Biosciences, Catalog No. 562426, RRID:AB_11152082), CD4 (BD Biosciences, Catalog No. 560650, RRID:AB_1727476), CD8

(BD Biosciences, Catalog No. 641400, RRID:AB_1645736), CD19 (BD Biosciences, Catalog No. 562653, RRID:AB_2722592), CD16 (BD Biosciences, Catalog No. 332779, RRID:AB_2868628), CD56 (BD Biosciences, Catalog No. 345812, RRID:AB_2629216), and CD14 (BD Biosciences, Catalog No. 641394, RRID:AB_1645725) antibodies. Regulatory T-cell subpopulations were analyzed using CD45, CD3, CD4, CD8, CD25 (BD Biosciences, Catalog No. 340907, RRID: AB_2819021), and CD127 (BD Biosciences, Catalog No. 560549, RRID:AB_1645486) antibodies. Proliferating T-cell subpopulations were analyzed using CD45, CD3, CD4, CD8, and Ki67 (BD Bios ciences, Catalog No. 558615, RRID:AB_647130) antibodies. Activated and memory T-cell subpopulations were analyzed using CD45, CD3, CD4, ICOS (BD Biosciences, Catalog No. 557802, RRID:AB_396878), CD38 (BD Biosciences, Catalog No. 562444, RRID:AB_11151894), CD45RA (BD Biosciences, Catalog No. 550855, RRID:AB_398468), CD45RO (BD Biosciences, Catalog No. 555492, RRID:AB_395883), CCR7 (BD Biosciences, Catalog No. 560765, RRID:AB_2033949), and HLA-DR (BD Biosciences, Catalog No. 347403, RRID:AB_2868848) antibodies in different combinations. B-cell subpopulations (including ABC subsets) were analyzed using CD45, CD19, IgD (BD Biosciences, Catalog No. 555778, RRID:AB_396113), CD27 (BD Biosciences, Catalog No. 337169, RRID:AB_647368), CD38, CD80 (BD Bios ciences, Catalog No. 564160, RRID:AB_2738632), and CD86 (BD Biosciences, Catalog No. 555665, RRID:AB_396019) antibodies.

Cell-of-origin (COO) and genetic subtyping of DLBCL. COO subtyping was conducted using one of two methods: (i) local IHC methods by the investigators (10–12) or (ii) gene expression profiling of formalin-fixed, paraffin-embedded (FFPE) tumor samples using NanoString (Interpace Pharma Solutions) as described previously (13).

Recent studies have shown that, based on genetic alterations, it is possible to divide DLBCL into genetic subtypes beyond COO and that these subgroups differ in terms of prognosis and potential response to targeted agents (14–16). Thus, genomic alterations were determined and monitored at screening and longitudinally using FFPE tumor samples and cell-free DNA (cfDNA) from plasma collections. We used a custom-designed 597-gene targeted next-generation sequencing (NGS) panel (AZHeme 600) for mutation calling in combination with whole-genome sequencing (WGS) for low-pass copy-number analysis (17). Genetic subtypes of DLBCL were determined at baseline and longitudinally by the LymphGen classification tool using available tumor tissue and cfDNA samples (14).

STAT3 protein (BD Biosciences, Catalog No. 4904, RRID: AB_331269) and ASO expression were assessed by IHC in available and evaluable paired tumor samples collected at screening and C1D15. When positivity of cells was observed upon IHC staining, the pathologist estimated the number of positive lymphoid cells relative to the total population of lymphoid cells.

NGS: circulating tumor DNA (ctDNA). For ctDNA analysis and correlation with clinical response, plasma from blood samples was collected during screening, predose on Days 1, 8, and 15 of Cycle 1, and at every tumor assessment visit (every 8 weeks after Cycle 2), and as soon as possible after disease progression and treatment discontinuation. First, cfDNA levels in plasma were measured. Then, ctDNA analysis was conducted using a custom next-generation targeted sequencing panel (AZHeme 600) for mutation calling and WGS for copy-number analysis (17). Sample preparation, sequencing, and LymphGen classification was performed (17). For WGS, the average achieved depth was 20× in plasma and 31× in tissue samples; for the AZHeme 600 panel, the achieved depth was 1,305× in plasma and 983× in tissue samples.

Gene expression profiling. Peripheral blood was analyzed using NanoString immunoprofiling for target gene expression in peripheral blood mononuclear cells. Total RNA from the peripheral blood cells collected in PAXgene blood RNA tubes was used for gene expression analysis using NanoString and PanCancer IO 360 Panel (770 genes).

Statistical analyses

The planned study sample size was 21 patients, which depended on the first 6 patients passing the DLT assessment after completing Cycle 1. An interim analysis for futility was performed after 10 sequentially enrolled patients had completed at least two on-treatment efficacy assessments. The futility boundary was defined as a less than 10% probability for the ORR to be above the target response rate of 40% for R/R DLBCL. Safety analyses included all patients who received at least one dose of the study treatment. Efficacy analyses included all patients in the safety analysis set who had a baseline tumor assessment and did not cross over from another arm. Statistical analyses for exploratory outcomes were not performed for the comparison of responders and nonresponders due to small sample size.

Availability of materials and data

Data underlying the findings described in this article may be obtained in accordance with AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure.

Data for studies directly listed on Vivli can be requested through Vivli at www.vivli.org. Data for studies not listed on Vivli can be requested through Vivli at https://vivli.org/members/enquiries-about-studies-not-listed-on-the-vivli-platform/. AstraZeneca Vivli member page is also available outlining further details: https://vivli.org/ourmember/astrazeneca/.

Results

Study population

A total of 17 patients were enrolled and treated with the acalabrutinib and danvatirsen combination regimen (**Table 1**). The median age was 72 (range, 34–88) years, and 53% (9/17) of patients were ages ≥65 years. The median number of prior therapies was 2 (range, 1–6). The most common prior systemic therapies were rituximab (94%), cyclophosphamide (88%), and vincristine (82%; Supplementary Table S2). At study entry, 82.4% of patients had stage IV disease; 35.3% were refractory to the last antilymphoma treatment and 64.7% relapsed after initial response. Of note, 11.8% and 17.6% had high-grade B-cell lymphomas or transformed DLBCL, respectively. At the data cutoff date of March 31, 2021, the median time on study was 10.2 months (range, 0.4–23.7), and all patients had discontinued treatment. Patients discontinued acalabrutinib and danvatirsen due to progressive disease [PD; 65% (11/17) and 59% (10/17), respectively], AEs [18% (3/17) and 24% (4/17)], and investigator decision [18% (3/17) and 18% (3/17)]. Notably, 1 patient discontinued danvatirsen due to an AE and then discontinued acalabrutinib due to PD within 9 days. The subsequent treatments following therapy discontinuation are listed in Supplementary Table S3; 1 patient had CAR-T therapy post-treatment discontinuation.

Table 1. Patient demographics and baseline characteristics.

Characteristic	Acalabrutinib + Danvatirsen (*N* = 17)
Age, median (range), years	72 (34–88)
<65 years	8 (47)
≥65 years	9 (53)
Male sex	12 (71)
ECOG PS	
0	5 (29)
1	10 (59)
2	2 (12)
No. prior lines of therapy, median (range)	2 (1–6)
Prior stem cell transplant[a]	3 (18)
Prior CAR-T therapy	3 (18)
Lugano classification	
III	2 (12)
IV	14 (82)
Missing	1 (6)
Histology	
DLBCL associated with chronic inflammation	1 (6)
DLBCL, nongerminal center type	1 (6)
Transformed DLBCL	3 (18)
De novo DLBCL, not otherwise specified	8 (47)
High-grade B-cell lymphoma[b]	2 (12)
T-cell/histiocytic-rich large B-cell lymphoma	2 (12)

Note: Data are *n* (%) unless otherwise specified.
[a]One patient had autologous stem cell transplantation, and 2 patients had allogeneic stem cell transplantation.
[b]With rearrangements of *MYC* and *BCL2* or of *BCL6* and *MYC*.

Primary endpoint: safety

The most commonly reported AEs of any grade, regardless of relationship to study treatment, were anemia (53%), increased alanine aminotransferase (ALT; 47%), decreased platelet count (47%), increased aspartate aminotransferase (AST; 41%), fatigue (35%), and decreased neutrophil count (29%; **Table 2**). Only 1 patient (6%) experienced febrile neutropenia (Grade 3). One DLT of Grade 3 (increased ALT/AST) was observed. ALT and AST elevations were transient in nature, and at data cutoff, the Grade 3 ALT/AST elevation was reported as resolved. AEs that led to discontinuation of acalabrutinib were lower gastrointestinal hemorrhage, edema, and platelet count decreased, each occurring in 1 patient; AEs that led to discontinuation of danvatirsen were ALT increased, lower gastrointestinal hemorrhage, edema, and platelet count decreased, each occurring in 1 patient. There were no treatment-related deaths.

Secondary endpoints

Clinical response. ORR was 23.5% ($n = 4/17$); 2 patients (11.8%) achieved CR and 2 (11.8%) achieved PR; median time to onset of response from first dose was 1.8 months and median DOR was 1.9 months (**Fig. 1**). The durations of the two CRs were 1.9 and 3.7 months. Median PFS was 2.0 months [95% confidence interval (CI), 0.8–3.6], and median OS was 19.3 months (95% CI, 2.0–not estimable). Among the 2 patients with a CR, both of whom had received one prior line of therapy, 1 patient had previously received R-CHOP and methotrexate, and the other patient had previously received R-CHOP. Among the 2 patients with a PR, 1 patient had received five prior lines of therapy, most recently with pembrolizumab; this patient had also received prior CAR-T therapy. The other patient had received two prior lines of therapy, most recently with cisplatinum, cytarabine, etoposide, methylprednisolone, and rituximab. Of the remaining 13 patients, 2 patients (11.8%) had stable disease (SD) and 7 patients (41.2%) had PD.

Pharmacokinetics

The PK of acalabrutinib and danvatirsen were generally consistent with their historical monotherapy profiles; the PK of acalabrutinib was also consistent with the historical profile of its major metabolite, ACP-5862 (Supplementary Fig. S2).

Immunogenicity

No antidrug antibodies to danvatirsen were detected in any patient at any time during the study.

Exploratory assessments

COO and genetic subtype classification of DLBCL. Among the 6 patient samples assessed by IHC-based COO analysis, 2 patients were classified as germinal center B-cell like (GCB), and the remaining 4 patients were classified as non-GCB subtype DLBCL. On the basis of the NanoString COO analysis, 2 patients were classified as ABC subtype DLBCL (Supplementary Table S4). Of the 2 ABC patients, 1 had a best response of CR and 1 had PD. The 2 GCB patients had PD. Of the 4 non-GCB patients, 1 had CR, 1 had PR, 1 had SD, and 1 had PD as the best response. No clear correlation between DLBCL COO and best response was seen; however, it is worth noting that both GCB patients in this study were nonresponders, and among the 2 CRs, 1 was ABC and the other was non-GCB, and the PR patient with COO data was a non-GCB subtype DLBCL.

On the basis of sample availability, FFPE tumor samples from 3 patients collected at screening and cfDNA samples collected at screening and longitudinally during the treatment period from most of the patients were analyzed for genetic subtype analysis using the LymphGen algorithm. Analysis of the three screening FFPE tumor samples showed that 1 patient (patient #1) had BN2 subtype (patient #1 was also identified as ABC DLBCL subtype based on COO analysis); 2 patients had A53 (aneuploid with *TP53* inactivation) subtype. The BN2 subtype is known to have a favorable outcome among ABC cases (14). Indeed, patient #1 was 1 of 2 patients who achieved CR.

Analysis of cfDNA collected at screening revealed 1 patient with N1 (*NOTCH1* mutations) subtype, 5 patients with A53 subtype, 1 patient with MCD subtype (co-occurrence of $MYD88^{L265P}$ and *CD79B* mutations), and 1 patient with EZB (*EZH2* mutations and *BCL2* translocations)/A53 mixed subtype. On the basis of the analysis of cfDNA collected at screening, 5 patients could not be categorized into any LymphGen subtype and therefore were classified as "other" [these patients included 3 originally identified as BN2 (including patient #1), A53, and A53/other subtypes by screening FFPE tumor data].

LymphGen analysis results based on cfDNA collected at Cycle 1, Day 1 were consistent with the results based on cfDNA collected at screening, except for patient #10, who was subtyped as "other" based on screening cfDNA and as EZB subtype based on cfDNA collected at Cycle 1, Day 1. Some samples may have been identified as "other" using cfDNA due to lower resolution of cfDNA-based LymphGen analysis compared with the tumor tissue-based LymphGen analysis, especially when a targeted DNA sequencing panel was used to determine mutations.

Table 2. Most common AEs occurring in ≥10% of patients, regardless of relationship to study treatment.

	Acalabrutinib + Danvatirsen (*N* = 17)	
AE, *n* (%)	**Any grade**	**Grade ≥3**
Anemia	9 (53)	8 (47)
ALT increased	8 (47)	4 (24)
Platelet count decreased	8 (47)	4 (24)
AST increased	7 (41)	1 (6)
Fatigue	6 (35)	2 (12)
Neutrophil count decreased	5 (29)	4 (24)
Arthralgia	3 (18)	0
Epistaxis	3 (18)	1 (6)
Hypokalemia	3 (18)	1 (6)
Hypotension	3 (18)	0
Nausea	3 (18)	0
Neutropenia	3 (18)	1 (6)
Pyrexia	3 (18)	0
Thrombocytopenia	3 (18)	2 (12)
Vomiting	3 (18)	0
Blood bilirubin increased	2 (12)	1 (6)
Blood creatinine increased	2 (12)	0
Cough	2 (12)	0
Dehydration	2 (12)	0
Dyspnea	2 (12)	0
Fall	2 (12)	0
Hematuria	2 (12)	0
Headache	2 (12)	0
Hypoalbuminemia	2 (12)	1 (6)
Hypoxia	2 (12)	1 (6)
Peripheral edema	2 (12)	0
Pain in extremity	2 (12)	0
Pneumonia	2 (12)	0
Maculopapular rash	2 (12)	0
Skin infection	2 (12)	0

Notably, the second patient with CR was identified as having N1 subtype, and the 2 patients with PR were identified as having A53 and A53/other subtypes. Interestingly, *BN2* and *N1* subtypes seen in the patients with CR in this study are the two LymphGen subtypes known to have significant upregulation of *NOTCH* target genes. These data show that all responders in the study had either *BN2*, *N1*, or *A53* subtype, which are known to potentially respond to BTK inhibitors (14, 18).

Among the patients with PD were the sole patient with MCD (another subtype that has been reported to respond to BTK inhibitors; ref. 14) and the sole patient with *EZB* subtype. Although classifications varied, a higher prevalence of *EZB*-related mutations was seen in nonresponders (Supplementary Fig. S3). More specifically, recurrent *KMT2D* and *CREBBP* mutations, copy-number alterations in chromosome 21, and fusions in *BCL2* were observed. Notably, both patients with CR had *ARID1A* mutations.

Results of STAT3 protein and ASO expression via IHC were available for 3 patients with paired samples (Supplementary Fig. S4). ASO was detected in Cycle 1, Day 15 samples from patients with reduced STAT3 protein expression.

Plasma cfDNA concentrations correlate with prognosis

In 4 of 6 PD patients with available cfDNA data, baseline and longitudinal plasma cfDNA concentrations were higher than in the 2 non-PD patients (**Fig. 2A**). Plasma cfDNA concentrations showed a gradual increase over time in these 4 PD patients. Both CR patients had lower baseline and longitudinal plasma cfDNA concentrations than did 4 of the 6 PD patients with available cfDNA data.

Dynamics of mutations and copy-number alterations. Sequencing of screening FFPE tumor samples and screening and longitudinal cfDNA samples from plasma revealed different patterns of mutational frequencies in response to treatment. Tumor samples and cfDNA samples were available for all 17 patients for allele frequency analysis. The allele frequency of mutations within cfDNA showed a trend toward lower allele frequency in the 4 responders compared with the 9 nonresponders (**Fig. 2B**; Supplementary Fig. S5).

One patient (patient #1) achieved an early CR after treatment Cycle 2 (**Fig. 3A**), which correlated with dynamic changes in ctDNA (**Fig. 2B**), including the disappearance of copy-number changes (**Fig. 3B**) and an increase in cfDNA at Cycle 3, Day 1 before PD at Cycle 5, Day 1 (when initial target lesions were undetectable based on minimal residual disease assessment; **Fig. 3C**). The longitudinal mutation plot for this patient showed that, following treatment, the allele frequency of almost all mutations, including *BCR* and *TP53* mutations, decreased to under 10% after Cycle 1, Day 1 (**Fig. 2B**). The only mutation that did not follow this trend in this patient was in *ASXL1*, which has been commonly implicated in clonal hematopoiesis of indeterminate potential (CHIP; ref. 19) and was not detected in the FFPE tumor sample (further suggesting it is a CHIP mutation). A *NOTCH1* (*P2514fs*) mutation and a *TP53* mutation with a very low allele frequency at screening were observed in patient #2, who also achieved CR; both mutations were cleared after Cycle 1, Day 8 (Supplementary Fig. S5). *NOTCH1* and *TP53* mutations with more than 15% allele frequency were observed at screening in the FFPE sample of patient #3, who achieved PR (**Fig. 2B**). During the treatment period, no mutations with more than 5% allele frequency were detected in monitored genes (including *BCR*) in patients achieving PR (patient #3 and patient #4; **Fig. 2B**; Supplementary Fig. S5).

In general, most patients with PD ($n = 7$) had a higher mutation burden than non-PD patients ($n = 10$), as measured by the number of mutations and variant allele frequency in baseline tumor and baseline and longitudinal plasma samples (**Fig. 2B**; Supplementary Fig. S5). Most of the variant allele frequencies for mutations detected in patients with SD or PD ($n = 9$) at screening and Cycle 1, Day 1 did not decrease at later visits during the study. Moreover, *KMT2D* and *CREBBP* mutations and *BCL2* fusions were detected in some patients with SD or PD ($n = 9$) but not in those with CR or PR ($n = 4$) in this study.

Plasma samples were available for 13 patients for analysis of copy-number alterations. On the basis of analysis of baseline and longitudinal plasma samples using low-pass WGS, nonresponders (i.e., PD or SD as best response; $n = 7$) had markedly more copy-number alterations at baseline and at later visits during the study compared with responders ($n = 4$; **Fig. 4**; Supplementary Fig. S6). There was also a trend of increasing copy-number alterations over time in nonresponders ($n = 7$). The 2 patients with CR and 1 of the 2 patients with PR had fewer copy-number alterations in baseline samples, and some of these copy-number alterations disappeared at later visits. The other patient with PR had many copy-number alterations in baseline samples; however, most of these copy-number alterations were not detectable in later visits. The frequency of copy-number alterations also decreased in 1 patient with CR (patient #1) and 1 patient with PR (patient #3) gradually during the treatment period. However, the copy-number alterations and the allele frequencies of the monitored mutations in 2 patients with PD (patients #8 and #9) increased

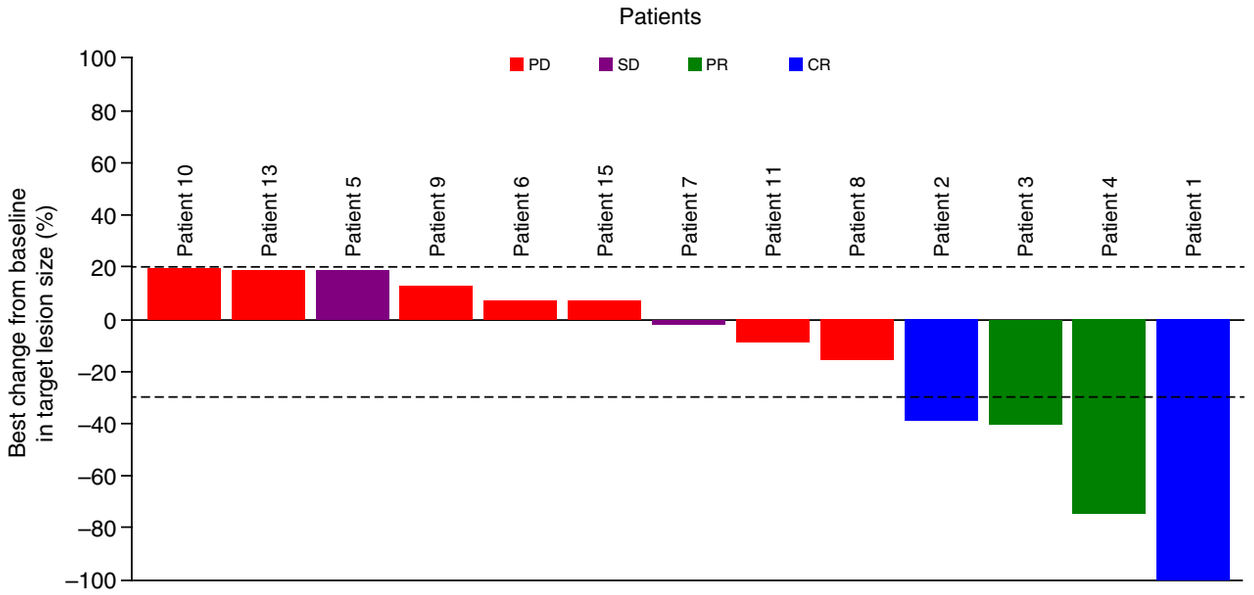

Figure 1.
Target lesion size (best percentage change from baseline) waterfall plot. Best change in target lesion size is the maximum reduction from baseline or the minimum increase from baseline in the absence of a reduction.

longitudinally. These data suggest that the allele frequency of mutations and frequency of copy-number alterations detected at screening decrease in responders and tend to remain stable or increase in nonresponders. The reduction in the copy number may reflect decreased tumor content. Nevertheless, patients with persistent copy number changes detected (≥1 alteration) as early as Cycle 1, Day 8 were less likely to respond to acalabrutinib in combination with danvatirsen.

Correlation of response and gene expression

On the basis of target gene expression analysis using a NanoString panel, comparison of baseline blood samples collected at Cycle 1, Day 1 predose revealed that expression of genes associated with $CD19^+$ B-cell signaling, such as *CD20* (MS4A1), *CD79A*, and *CD22*, was significantly higher in the 4 patients who achieved CR or PR versus the 6 patients who achieved SD or PD (Supplementary Fig. S7A). Analysis of gene expression in longitudinal peripheral blood samples also showed that expression of genes associated with B-cell signaling was higher in most responders and was maintained through the treatment period (Supplementary Fig. S7B). Nonresponders ($n = 9$) had very low numbers of circulating B cells and B-cell–related gene expression in peripheral blood at baseline (Supplementary Fig. S7C). The 6 patients who did not respond to therapy, however, had mostly higher levels of expression of genes in the IFNα signaling pathway, including *IL10*, *JAK2*, *SERPING1*, *CXCL10*, and *STAT2*, compared with the 4 patients who responded to the therapy at screening and during treatment (Supplementary Fig. S8A and S8B).

Immune cell profiles in peripheral blood samples

Immunophenotyping data showed higher frequencies of B cells in circulating lymphocyte fractions (Supplementary Fig. S9) in responders versus nonresponders at baseline. Interestingly, only responding patients (CR and PR) and 1 SD patient had moderate (2.9–5.6% of lymphocytes) to high (14.5–23.2% of lymphocytes) levels of B cells at Cycle 1, Day 1 predose. Patients with PD and the other patient with SD either did not have detectable B cells or had very low levels (<0.5% in the lymphocyte population). These data corroborate the results of the gene expression analysis showing higher B-cell–related gene expression in responders compared with nonresponders (Supplementary Fig. S7C). In addition, among responders, patients with PR had higher levels of peripheral B cells than patients with CR at all time points checked. The frequency and absolute numbers of NK cells and monocytes did not correlate with response to therapy (Supplementary Fig. S10A).

Assessment of T-cell and B-cell subpopulations (Supplementary Fig. S10A and S10B) showed that the frequency of naïve T cells with $CD3^+CD4^+CD45RA^+CD45RO^-CCR7^+$ phenotype in the $CD4^+$ T-cell population was lower in patients with PD than those with CR, PR, and SD at all but one time point evaluated.

Cytokine levels in plasma

In general, the pretreatment mean plasma levels of various cytokines and chemokines such as IL6, TNFα, IL10, IL16, and IFNγ were higher in patients with PD compared with other patients, both at baseline and at later visits (Supplementary Fig. S11).

Discussion

In this phase I clinical study, the combination of STAT3 ASO and the BTK inhibitor acalabrutinib was safe and tolerable for the treatment of R/R DLBCL, but the DOR was short. Four of 17 patients enrolled in this study responded to treatment (2 CR and 2 PR); 2 patients had SD, 7 patients had PD, and 4 patients were not evaluable. One main reason for differential response could be due to genetic differences between responders and nonresponders. DLBCL is a heterogeneous disease, and recent studies demonstrate that DLBCL

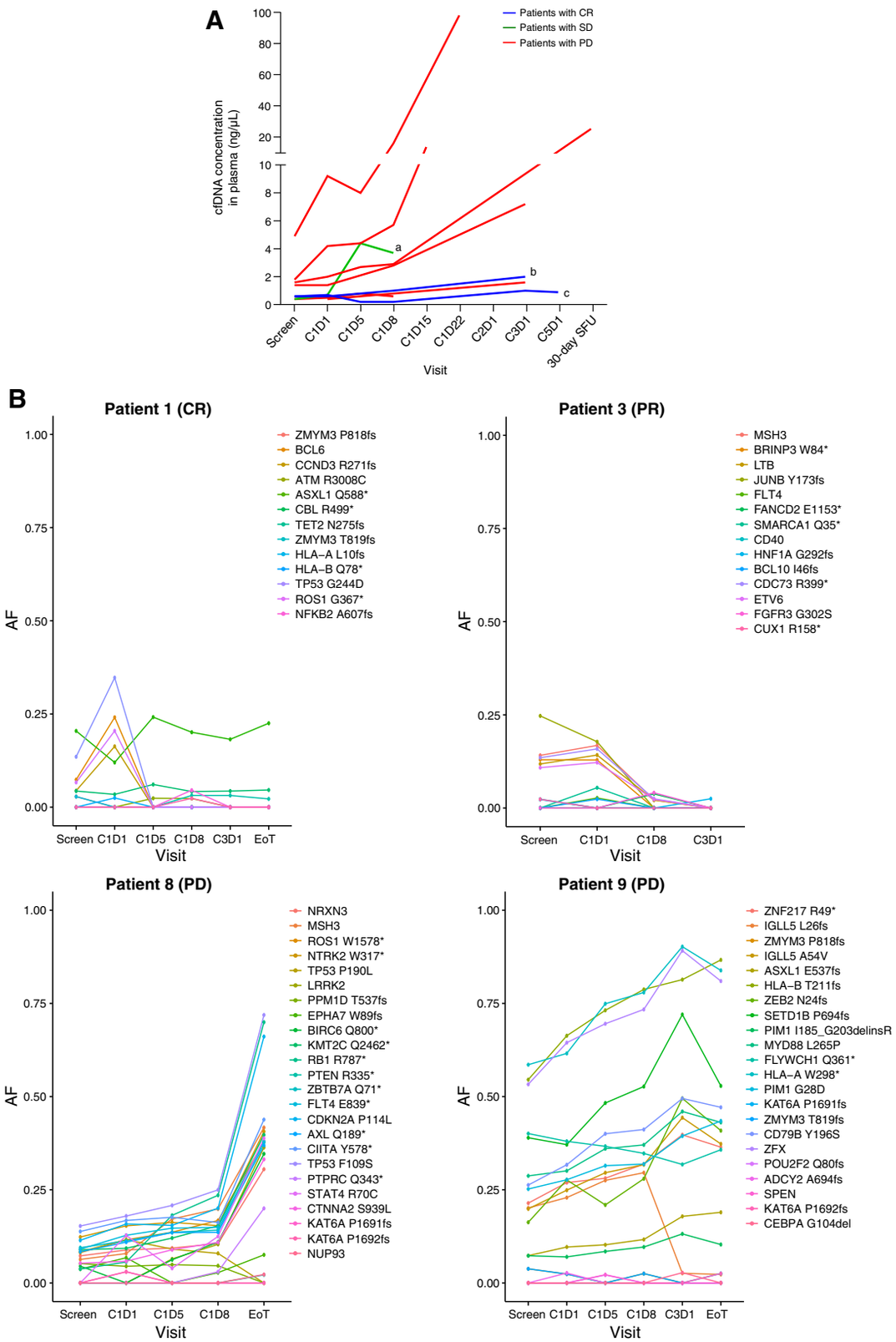

Figure 2.

Cell-free DNA concentrations in plasma (**A**) and variant allele frequency (**B**) over time by response. a, Patient 7 achieved SD on Day 52. b, Patient 2 achieved CR on Day 53. c, Patient 1 achieved CR on Day 54. C, cycle; D, day; SFU, safety follow-up.

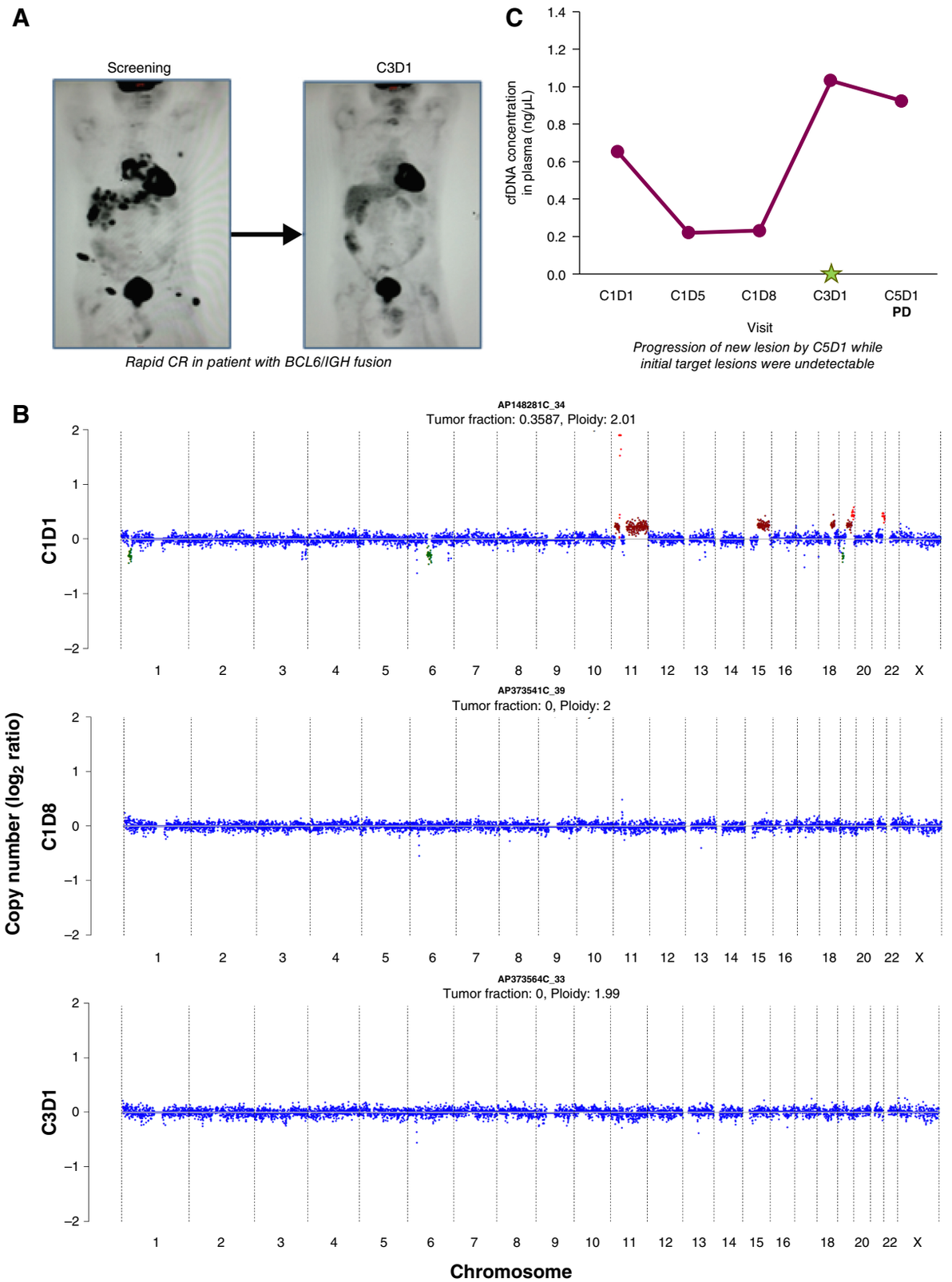

Figure 3.

Example patient with early CR (patient #1). **A,** PET/CT scan (screening and end C2); **B**, copy-number variations; **C**, cfDNA concentration in plasma. The green star indicates MRD negativity based on peripheral blood by clonoSEQ. C, cycle; D, day; MRD, minimal residual disease.

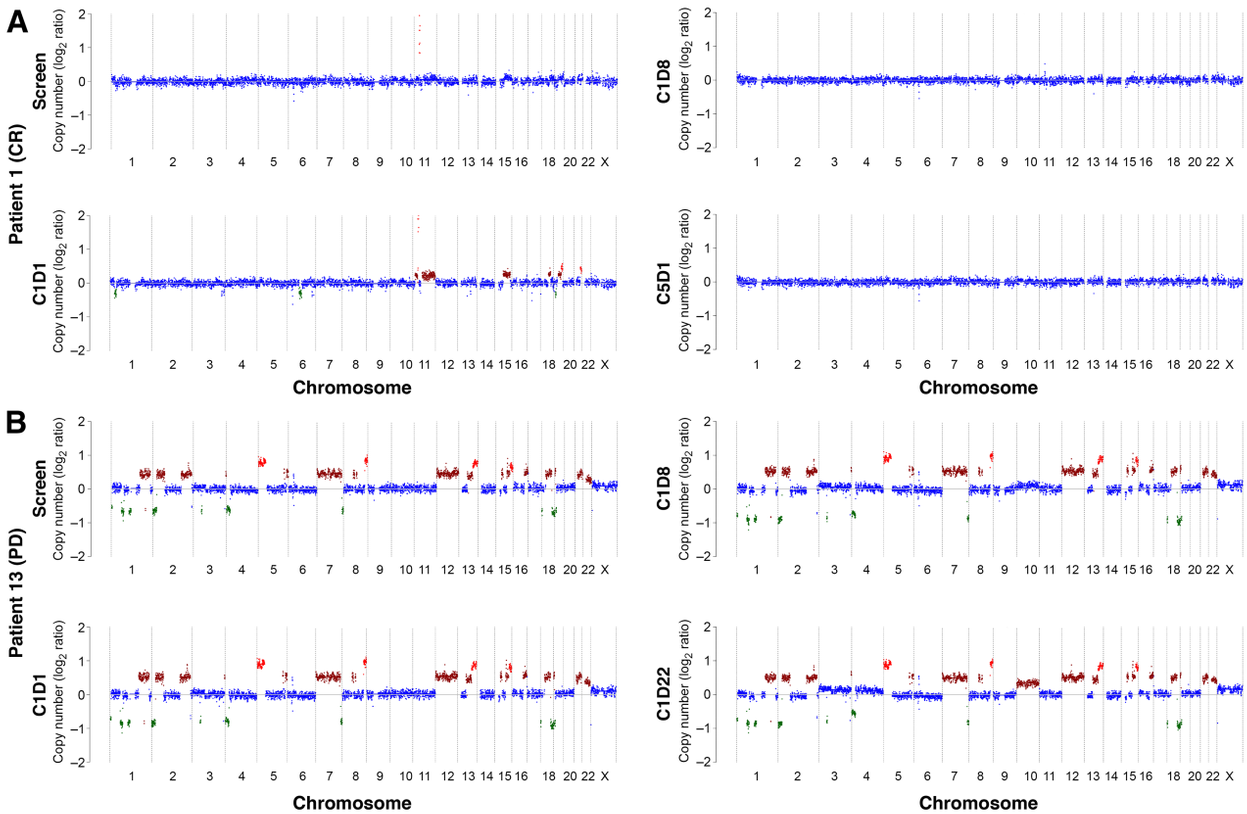

Figure 4.
Copy-number dynamics of CR (**A**) versus PD (**B**). AF, allele frequency; C, cycle; D, day.

has many genetic subtypes based on mutation status, with some subtypes showing correlation with response to certain therapies (14). It is important to note that all responders in this study were either BN2, N1, or A53, subtypes that are known to potentially respond to BTK inhibitors (14, 18). However, 1 patient with PD had MCD, a subtype that is also known to respond to BTK inhibitors (14, 18), suggesting that response in this study could have been affected by other factors such as age, previous treatments, or epigenetic mechanisms of BTK inhibitor resistance (20).

In our current study of patients treated with the combination regimen of acalabrutinib and danvatirsen, the most commonly reported Grade ≥3 AEs included hematologic and hepatic AEs, regardless of relationship to study treatment. Similarly, hematologic and hepatic AEs were the most frequently reported AEs from the phase Ib study of 30 patients with DLBCL treated with danvatirsen monotherapy (8). In our study, 47% of patients experienced Grade ≥3 anemia, whereas 3% of patients in the danvatirsen monotherapy study reported this AE (8). Increased ALT was reported in 24% of patients in our combination study compared with 7% of patients in the monotherapy study (8). Notably, in the danvatirsen monotherapy study (8), 10 of the 30 patients were treated with a lower dose than the recommended phase II dose (RP2D; 2 mg/kg instead of 3 mg/kg or 200 mg), whereas in our study, all 17 patients were treated with danvatirsen 200 mg. When pooling data from all patients treated with danvatirsen monotherapy at the RP2D (3 mg/kg or 200 mg), including 63 patients with DLBCL, hepatocellular carcinoma, advanced cancers, and solid tumors from four clinical studies, Grade ≥3 increased ALT/AST was 17.5%/12.7% (data on file).

In the efficacy-evaluable population in this study, and with the caveat of the small sample size ($n = 17$), modest clinical activity was observed (ORR, 23.5%; CR, 11.8%) with limited durability (median DOR, 1.9 months). These findings were similar to results seen with acalabrutinib monotherapy in 21 patients with R/R DLBCL (ORR, 24%; CR, 19%; ref. 3); however, our results were not as robust as those seen with the most recently approved therapies in patients with R/R DLBCL, including tafasitamab–lenalidomide ($n = 80$; ORR, 60%; CR, 43%; median DOR, 21.7 months; ref. 21); loncastuximab ($n = 145$; ORR, 48%; CR, 24%; median DOR, 10.3 months; ref. 22); selinexor ($n = 127$; ORR, 28%; CR, 12%; median DOR, 9.3 months; ref. 23); axicabtagene ciloleucel ($n = 101$; ORR, 83%; CR, 58%; ref. 24); and lisocabtagene maraleucel ($n = 256$; ORR, 73%; CR, 53%; ref. 25). Considering the totality of the safety and efficacy data and following discussion of the durability of response with the combination therapy, the investigators decided to permanently close module 1 to further enrollment.

JAK/STAT3 signaling can be activated by inactivation of the *SOCS1* or *DUSP2* genes and also by activating mutations in the *STAT3* gene (14). On the basis of the baseline mutation analysis using pretreatment tumor and Cycle 1, Day 1 cfDNA, none of the patients in this study had mutations in the *STAT3* or *DUSP2* genes. Loss of function (nonsense) mutations in the *SOCS1* gene were observed in 2 patients (patients #10 and #13) who experienced PD, suggesting that these 2 patients could have had elevated levels of JAK/STAT signaling. Nonetheless, these patients did not respond to the therapy, suggesting

that either the *STAT3* inhibition level was not efficient or BTK and *STAT3* co-inhibition was not sufficient to target and impair the malignancy. Another patient with PD (patient #9) was classified as MCD subtype, based on LymphGen analysis. MCD models are known to be dependent on *STAT3* signaling (14). Interestingly, this patient (patient #9) was also the only patient with the $MYD88^{L265P}$ mutation, a hallmark of MCD, a subtype associated with a relatively inferior survival (14). The limited number of available on-treatment paired tumor samples precluded definitive conclusions on *STAT3* expression in this study.

ABC patients have shown greater response to BTK inhibitors than GCB patients, who respond very poorly (4). The results of this study demonstrate that, of the 2 patients who achieved CR, 1 was an ABC subtype and the other was a non-GCB subtype. One patient with PR was also a non-GCB subtype, whereas both GCB patients were nonresponders.

Patients with EZB-related alterations (i.e., *KMT2D*/*CREBBP* mutations or *BCL2* fusion) are known to not respond to acalabrutinib-STAT3 inhibition (14). In our study, the only EZB patient was also a nonresponder (PD). However, both responders had *ARID1A* mutations. These findings are similar to those seen in a clinical trial evaluating the combination of acalabrutinib plus vistusertib (ACE-LY-110; ref. 17). Longitudinal samples showed that known mutations do not reduce to zero allele frequency following treatment in nonresponder patients.

Analysis of immunophenotyping and gene expression profiling data in responders revealed higher levels of circulating B cells and B-cell–related gene expression in peripheral blood (indicating a decreased B-cell population in nonresponders; Supplementary Figs. 7 and 9). These results are in line with a previous report that suggested that the reduction in B-cell levels in peripheral blood correlates with poor prognosis in DLBCL patients (26).

The findings from our analysis of cytokine and chemokine levels showed that nonresponders had higher plasma cytokine levels in general. Nonresponders also had higher expression of IL10 and IFNγ-related genes. Our findings corroborate previously published data and suggest that pretreatment and on-treatment levels of various cytokines and chemokines correlate with prognosis and are increased in DLBCL patients with poor outcomes (27–29).

cfDNA concentration in plasma has been reported to be increased in DLBCL patients compared with healthy controls (30–33). High cfDNA concentration among DLBCL patients was also shown to correlate with worse outcomes (31, 33), and higher cfDNA at diagnosis was shown to correlate with response to therapy (34). In this study, 4 of 6 PD patients with available cfDNA data had higher baseline plasma cfDNA concentration compared with other patients. The cfDNA concentration in plasma in these 4 PD patients also increased over time and reached higher levels compared with baseline levels.

Recently, ctDNA levels in DLBCL patients before treatment and changes in ctDNA levels during treatment were also shown to be two independent prognostic factors for survival outcomes (35). In that analysis, higher pretreatment ctDNA levels correlated with reduced event-free survival and OS, and a significant reduction in ctDNA levels during treatment correlated with both response and prognosis (35). Similarly, another recent study showed that baseline ctDNA levels correlated with some known clinical risk factors and prognosis in R/R DLBCL; patients with high baseline ctDNA levels had shorter PFS and OS (36). Patients with CR had greater decrease in median ctDNA levels than patients with PD at the end of treatment. Although ctDNA levels were not measured in this study, nonresponders (SD and PD) in general had higher mutation burden compared with responders (CR and PR) as assessed by the number and allele frequencies of mutations and copy-number alterations. These published data and the data from this study suggest that cfDNA and ctDNA levels may correlate with disease status and outcome in DLBCL.

This study is limited by the very small number of patients, which may not appropriately represent the general population of patients with DLBCL (Supplementary Table S5). In addition, the results should be interpreted with caution due to the small sample size. Interpretation of COO results is further limited by the use of different assessment modalities (IHC or Nanostring). Definitive conclusions cannot be drawn to associate response outcomes with cfDNA levels and immune B-cell fraction due to the very limited sample size. However, the data from this study suggest that certain biomarkers such as baseline and longitudinal cfDNA and cytokine levels in plasma, circulating B-cell levels, and mutation burden could be used to predict outcome in R/R DLBCL.

In conclusion, results from this arm of the PRISM study suggest that targeting BTK and STAT3 is safe and tolerable in patients with relapsed DLBCL but has limited efficacy. Although no definitive conclusions can be drawn regarding associations between lower cfDNA levels/mutation burden and clinical responses in this study, findings support further exploration of these potential biomarkers in studies with targeted agents for patients with DLBCL. cfDNA levels and mutational burden are known prognostic molecular biomarkers of DLBCL (37); however, it is uncertain at this time whether they are also possible predictive biomarkers for this specific combination therapy. Findings from this study may help inform future studies in DLBCL to explore correlative analyses and their association to clinical responses.

Authors' Disclosures

M.R. Patel reports other support from AstraZeneca during the conduct of the study. P.M. Reagan reports grants from Seagen and Genentech as well as personal fees from Kite Pharma and Caribou Biosciences outside the submitted work. N.S. Saba reports personal fees from AbbVie, Janssen, BeiGene, Genentech, Kite Pharma, Karyopharm, Epizyme, and ADC Therapeutics outside the submitted work. G. Collins reports personal fees from Roche, Takeda, Gilead, and Novartis; grants and personal fees from Beigene and Astra Zeneca as well as grants from BMS and Amgen outside the submitted work. T. Arkenau reports grants from Sarah Cannon Research Institute during the conduct of the study. B.R. Nuttall reports employment with AstraZeneca. M. Acar reports employment with AstraZeneca. R.D. White reports other support from AstraZeneca during the conduct of the study as well as other support from AstraZeneca outside the submitted work. M. Udriste reports other support from AstraZeneca outside the submitted work. S. Sharma reports other support from AstraZeneca outside the submitted work and reports employment with AstraZeneca and ownership of AstraZeneca stock. B.A. Dougherty reports other support from AstraZeneca during the conduct of the study as well as other support from AstraZeneca outside the submitted work and also has a patent for AstraZeneca issued. A. Mortlock reports other support from AstraZeneca during the conduct of the study as well as other support from AstraZeneca outside the submitted work. A. Forcina reports other support from AstraZeneca during the conduct of the study. V. Munugalavadla reports other support from AstraZeneca and Gilead Sciences outside the submitted work. I.W. Flinn reports grants from AstraZeneca during the conduct of the study; grants and other support from AbbVie, BeiGene, Genentech, InnoCare Pharma, Kite Pharma, Myeloid Therapeutics, Novartis, TG Therapeutics, and Vincerx Pharma; grants from Acerta Pharma, Agios, ArQule, AstraZeneca, Biopath, Bristol Myers Squibb, CALIBR, CALGB, Century Therapeutics, City of Hope National Medical Center, Constellation Pharmaceuticals, Curis, CTI Biopharma, Epizyme, Fate Therapeutics, Forma Therapeutics, Forty Seven, Gilead Sciences, IGM Biosciences, Incyte, Infinity Pharmaceuticals, Janssen, Loxo, Marker Therapeutics, Merck, Millennium Pharmaceuticals, and MorphoSys; other support from Celgene, Genmab, Hutichson MediPharma, Nurix, Pfizer, Pharmacyclics, Portola Pharmaceuticals, Rhizen Pharmaceuticals, Roche, Seattle Genetics, Step Pharma, Tessa Therapeutics, Trillium Therapeutics, Triphase Research & Development Corp., Unum Therapeutics, Verastem, and 2seventy

Bio; other support from Secura Bio, Servier Pharmaceuticals, and Xencor outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

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Acknowledgments

This study was sponsored and funded by AstraZeneca. AstraZeneca was responsible for conducting the mutation and tumor IHC assays and for analyzing the data. The authors thank Raquel Izumi, Ahmed Hamdy, Melanie Frigault, Graham Brock, Ed Clark, Gary de Jesus, and Joe Ware for their contributions to this study. Medical writing assistance, funded by AstraZeneca, was provided by Kim Tran-Kerr, PhD, MD, and Cindy Gobbel, PhD, of Peloton Advantage, LLC, an OPEN Health company, under the direction of the authors.

The publication costs of this article were defrayed in part by the payment of publication fees. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Received August 11, 2022; revised January 16, 2023; accepted June 21, 2023; published first June 26, 2023.

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