bjh research paper

Fimepinostat (CUDC-907) in patients with relapsed/refractory diffuse large B cell and high-grade B-cell lymphoma: report of a phase 2 trial and exploratory biomarker analyses

Daniel J. Landsburg,[1] Stefan K. Barta,[1,2] Radhakrishnan Ramchandren,[3,4] Connie Batlevi,[5] Swaminathan Iyer,[6,7] Kevin Kelly,[8] Ivana N. Micallef,[9] Sonali M. Smith,[10] Don A. Stevens,[11] Mariano Alvarez,[12] Andrea Califano,[12] Yao Shen,[12] Gideon Bosker,[12] Jefferson Parker,[13] Raul Soikes,[13] Elizabeth Martinez,[13] Reinhard von Roemeling,[13] Robert E. Martell[13] and Yasuhiro Oki[6,14]

[1]University of Pennsylvania, [2]Fox Chase Cancer Center, Philadelphia, PA, [3]University of Tennessee Knoxville, Knoxville, TN, [4]Karmanos Cancer Institute, Detroit, MI, [5]Memorial Sloan Kettering Cancer Center, New York, NY, [6]MD Anderson Cancer Center, [7]Houston Methodist Hospital, Houston, TX, [8]University of Southern California, Los Angeles, CA, [9]Mayo Clinic, Rochester, MN, [10]University of Chicago, Chicago, IL, [11]Norton Cancer Institute, Louisville, KY, [12]DarwinHealth, Inc, New York, NY, [13]Curis, Inc, Lexington, MA, and [14]Genentech, San Francisco, CA, USA

Received 20 May 2021; accepted for publication 13 July 2021

Correspondence: Daniel J. Landsburg, 3400 Civic Center Blvd, #12-182, Philadelphia, PA 19104, USA.
E-mail: daniel.landsburg@pennmedicine.upenn.edu

Presented in part at the 60th American Society of Hematology Annual Meeting and Exposition, December 2018, San Diego, CA, USA.

Summary

Fimepinostat (CUDC-907), a first-in-class oral small-molecule inhibitor of histone deacetylase and phosphatidylinositol 3-kinase, demonstrated efficacy in a phase 1 study of patients with relapsed/refractory (R/R) diffuse large and high-grade B-cell lymphomas (DLBCL/HGBL), particularly those with increased MYC protein expression and/or *MYC* gene rearrangement/copy number gain (MYC-altered disease). Therefore, a phase 2 study of fimepinostat was conducted in this patient population with 66 eligible patients treated. The primary end-point of overall response (OR) rate for patients with MYC-IHC ≥40% (n = 46) was 15%. Subsequently, exploratory pooled analyses were performed including patients treated on both the phase 1 and 2 studies based upon the presence of MYC-altered disease as well as a biomarker identified by Virtual Inference of Protein activity by Enriched Regulon analysis (VIPER). For these patients with MYC-altered disease (n = 63), the overall response (OR) rate was 22% with seven responding patients remaining on treatment for approximately two years or longer, and VIPER yielded a three-protein biomarker classification with positive and negative predictive values of ≥85%. Prolonged durations of response were achieved by patients with MYC-altered R/R DLBCL/HGBL treated with single-agent fimepinostat. Combination therapies and/or biomarker-based patient selection strategies may lead to higher response rates in future clinical trials.

Keywords: diffuse large B-cell lymphoma, MYC, histone deacetylase inhibitor, phosphatidylinositol 3-kinase inhibitor, biomarker.

Introduction

MYC is a human proto-oncogene which serves as a transcription factor regulating the control of cellular activities, particularly cell cycle activation.[1,2] In diffuse large B-cell lymphoma (DLBCL) and high-grade B-cell lymphoma (HGBL), described *MYC* abnormalities include rearrangement/translocation and copy number gain/amplification which are

First published online 2 August 2021
doi: 10.1111/bjh.17730

detected by fluorescence *in-situ* hybridization (FISH). *MYC* translocation/rearrangement has been shown to predict for inferior survival in patients with newly diagnosed DLBCL when treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP)[3,4] as well as those with R/R DLBCL following receipt of salvage immunochemotherapy with or without subsequent high-dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT).[5] Additionally, increased copy number of *MYC* is also associated with a poor prognosis following receipt of first-line immunochemotherapy.[6,7] Independent of *MYC* abnormalities, increased expression of MYC protein by immunohistochemical staining (IHC) is also predictive of inferior survival in patients with newly diagnosed DLBCL when treated with R-CHOP[7,8] as well as in the multiply relapsed/refractory setting.[9] Approximately 1/3 of newly diagnosed DLBCL/HBGL patients' tumours harbour the "MYC alterations" of *MYC* rearrangement/translocation and/or expression of MYC protein ≥40% by IHC,[10] and although the frequency of MYC alterations in R/R DLBCL/HGBL patient tumours has not been characterized well, it is likely that a higher proportion would be classified as MYC-altered given the high probability of treatment failure as mentioned above.

Fimepinostat (CUDC-907) is a first-in-class oral small-molecule inhibitor of histone deacetylase (HDAC) class I and II as well as phosphatidylinositol 3-kinase (PI3K) α, β and δ enzymes. Overall response rates of approximately 20–30% have been experienced by R/R DLBCL patients treated with HDAC inhibitors[11,12] and PI3K inhibitors.[13,14] With regard to *MYC*, HDAC inhibition leads to decreased transcription of *MYC* and translation of MYC messenger ribonucleic acid (mRNA) while PI3K inhibition leads to enhancement of ubiquitin-mediated MYC protein degradation, and treatment with fimepinostat has resulted in superior preclinical activity in DLBCL xenografts with MYC alterations, as compared to treatment with HDAC or PI3K inhibitor monotherapy.[15] Additional support of fimepinostat mechanistically targeting MYC activity has been demonstrated through Virtual Inference of Protein activity by Enriched Regulon analysis (VIPER) analysis of RNASeq profiles, which, after perturbation with 400 compounds in 19 cell lines, identified HDAC and PI3K inhibitors as two of the strongest classes of compounds in terms of MYC activity inhibition (Andrea Califano, personal communication). This may be due to increased expression and nuclear localization of the tumour suppressor protein FOXO1 by HDAC inhibition and PI3K inhibition respectively.[16] *FOXO1* is known to inhibit multiple target genes of *MYC in vitro*,[17] and the absence of *FOXO1* promotes lymphomagenesis by reducing *MYC*-induced apoptosis *in vivo*.[18]

Fimepinostat was first studied in patients with multiply R/R lymphoma or multiple myeloma in the phase 1 setting with the primary objective to determine the recommended phase 2 dose, which was 60 mg by mouth five days on/two days off.[19] A subgroup analysis of 11 evaluable DLBCL/HGBL patients with MYC-altered disease as defined by central or local testing demonstrated a 64% overall response (OR) rate and estimated 13·6 months duration of response.[20] Based upon these results as well as those from preclinical experiments, a phase 2 protocol of fimepinostat for patients with multiply R/R DLBCL/HGBL was developed, with classification of patients based upon MYC alteration status.

Here we report outcomes of patients treated in the phase 2 protocol, as well as an exploratory analysis of MYC-altered patients treated on the phase 1 and 2 protocols, in an effort to identify patients who derived clinical benefit from treatment with fimepinostat and help guide the design of future clinical trials with this agent.

Patients and methods

Included patients in the primary analysis were enrolled in the multicentre, multinational, open-label, single-arm phase 2 study to evaluate the efficacy and safety of CUDC-907 in patients with R/R diffuse large B-cell lymphoma, including patients with MYC alterations (NCT02674750). Patients enrolled on this study were treated on protocol from July 2016 through May 2019 in centres located in the United States, Spain and France. Key inclusion criteria were ≥18 years of age with histopathologically confirmed diagnosis of DLBCL, HGBL or transformed follicular lymphoma refractory to or relapsed after 2–4 prior lines of therapy for the treatment of *de novo* DLBCL and ineligible for (or failed) autologous or allogeneic stem cell transplant (SCT). Additional protocol information is available in the Protocol supplemental file. This protocol was approved by the institutional review boards of all participating centres and conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonization Good Clinical Practice guidelines, applicable regulatory requirements, and Curis policies.

Patients received fimepinostat (CUDC-907) capsules (Pharmatek Laboratories Inc., San Diego, CA, USA) orally, within 30 min of a meal, in 21-day cycles until disease progression was documented or other discontinuation criteria were met. Fimepinostat 60 mg by mouth five days on/two days off was the starting dose, changes in dose and/or schedule intensity of fimepinostat were allowed as per protocol due to toxicity. Safety and tolerability were assessed by the incidence and severity of adverse events as determined by the NCI Common Terminology Criteria for Adverse Events (CTCAE v4.03). The intention-to-treat (ITT) population included patients who met inclusion criteria and received at least one dose of fimepinostat. The evaluable patient population included all patients who received at least one full cycle of study treatment and had at least one post-baseline disease assessment.

MYC-altered disease was defined as one or more of the following results from central testing of tumour samples: expression of MYC protein in ≥40% of lymphoma cells by IHC (MYC-IHC), *MYC* rearrangement by FISH (MYC-R) or >2 copies of *MYC* by FISH (MYC-CN). Central testing

included IHC staining of MYC (rabbit clone Y69) and BCL2 (mouse clone 124) as well as FISH with *MYC* (8q24) and *BCL6* (3q27) break-apart probes and *BCL2* [t(14;18)] fusion probe performed by NeoGenomics Laboratories, Inc (Fort Myers, FL, USA), with positive cut-off values for *MYC* rearrangement (>10%), *MYC* copy number gain (>20%), *BCL2* rearrangement (≥0·5%) and *BCL6* rearrangement (>10%) as defined per laboratory standard. Cell of origin was defined as per Hans algorithm[21] by local testing.

Patients were prospectively classified into three categories based upon the presence or absence of MYC alterations. Group A was characterized by the presence of MYC-R or MYC-CN without MYC-IHC, Group B as MYC-IHC with or without MYC-R and/or MYC-CN and Group C as no MYC alterations identified by central testing or central testing unable to be performed due to lack of adequate tissue.

The primary objective was to determine the OR rate for Group B patients as per central radiographic review. Key secondary objectives were to determine the OR rate by local radiographic review, complete response (CR) rate, progression-free survival (PFS), overall survival (OS), disease control (DC) rate and duration of response (DOR) for Group B patients, determine the OR rate for Groups A and C and to evaluate the incidence and severity of adverse events (AE). Radiographic responses to treatment were made according to the Revised Response Criteria for Malignant Lymphoma.[22] Disease progression could also be defined by the investigator after consideration of clinical or laboratory features in the absence of diagnostic imaging. Survival times were estimated via the Kaplan–Meier method and 95% confidence intervals (CI) calculated via the binomial exact method. All statistical analyses were performed using Stata version 13 (StataCorp, College Station, TX, USA). A clinically meaningful OR rate was determined to be 30% with a sample size of 100 patients enrolled in Group B. An interim analysis was planned to occur when 50 patients had been enrolled in Group B, of which at least 25 patients were considered evaluable per protocol, with the lower bound of the 95% CI for OR to exceed 10% in evaluable Group B patients for the study to continue enrolment.

For the exploratory analysis of patients with MYC-altered disease, included patients were those from the evaluable population of the phase 1 protocol and the ITT population of the phase 2 protocols with MYC alterations as defined by central testing, or local testing in cases for which central testing for all of the following MYC alterations was not performed.

For the exploratory analysis of protein-based classifiers, RNASeq profiles from pre-treatment biopsies of 22 patients enrolled in the phase 1 and 2 trials were generated by Illumina sequencing. Protein activity was measured by VIPER analysis, which converts tumour sample gene expression profiles into accurate protein activity profiles for approximately 6 213 regulatory proteins, based on the expression of their transcriptional targets (DarwinHealth).[23] Unlike raw gene expression, VIPER-inferred protein activity is extremely reproducible, and this methodology (DarwinOncoTarget algorithm) has been approved by the NYS Department of Health CLIA/CLEP Validation Unit as an offering in the category of "Molecular and Cellular Tumour Markers for Oncology"[24] and shown to be effective for biomarker discovery.[25] The activity of 6 213 regulatory proteins annotated as Transcription Factors (GO:0003700, or GO:0004677 and GO:0030528 or GO:0045449) or co-Transcription Factors (GO:0003712 or GO:0030528 or GO:0045449) or signaling proteins (GO:0007165 and GO:0005622 or GO:0005886) in the Gene Ontology[26] was inferred by metaVIPER,[27] using transcriptional regulatory networks (interactomes) inferred by analysis of a DLBCL and an acute myeloid leukemia (AML) cohorts using the ARACNe algorithm.[28] MetaVIPER is an extension of the VIPER algorithm supporting integration of multiple regulatory networks. A fimepinostat-sensitivity classifier was generated by training a Neural Network[29] using the top $k = 1, \ldots, 10$ most differentially active proteins between responders and non-responders samples. The data set is available in Gene Expression Omnibus accession GSE171806.

Results

Seventy patients were enrolled on the phase 2 protocol, with four patients excluded from analysis due to never having been dosed with fimepinostat (2) or lacking confirmation of receipt of 2–4 lines of prior therapy (2), resulting in 66 patients included in the ITT population.

Baseline characteristics of the ITT population are described in Table I. Response and survival outcomes are described in Table II and are based upon local radiographic review. The OR rate for Group B patients ($n = 46$) was 15% (95% CI 6–29%) and the OR rate for all patients ($n = 66$) was 12% (95% CI 5–22%). Of note, seven out of eight responding patients were in Group B. Additionally, two responding patients proceeded to autologous stem cell transplantation. For all patients, the median time to response was 2·6 months.

Treatment-emergent adverse events (TEAE) occurring per patient by highest grade experienced with a frequency of ≥10% are listed in Table III. Three patients experienced a grade 5 TEAE: Guillain–Barré syndrome deemed unlikely related to treatment in one patient, sepsis deemed not related to treatment in one patient and tracheal obstruction deemed not related to treatment in one patient. One patient discontinued treatment due to grade 2 vomiting deemed related to treatment.

Enrolment onto the phase 2 protocol was stopped in August 2017 due to inconclusive efficacy as determined at the time of interim analysis, at which point the OR rate for evaluable Group B patients ($n = 28$) was 25% (95% CI 11–45%). In addition, central radiographic review was not subsequently performed.

For the exploratory analysis of patients with MYC-altered disease in the phase 1 and 2 protocols, 63 patients were

Table I. Baseline characteristics of phase 2 intention-to-treat and phase 1/2 MYC-altered patient populations.

Characteristic	Phase 2 ITT (n = 66) n (%)	Phase 1/2 MYC-altered (n = 63) n (%)
Age (median)	64 years	64 years
Age >60		
No	25 (38)	24 (38)
Yes	41 (62)	39 (62)
Sex		
Female	28 (42)	27 (41)
Male	38 (58)	36 (59)
ECOG score		
0–1	6 (92)	58 (92)
2	5 (8)	5 (8)
Stage		
I–II	10 (15)	9 (14)
III–IV	56 (85)	53 (84)
Unknown	0 (0)	1 (2)
LDH > upper limit of normal		
No	24 (36)	25 (40)
Yes	42 (64)	37 (59)
Unknown	0 (0)	1 (1)
Extranodal disease >1 site		
No	32 (48)	35 (56)
Yes	34 (52)	27 (43)
Unknown	0 (0)	1 (1)
International Prognostic Index Score >2		
No	24 (36)	26 (41)
Yes	42 (64)	34 (54)
Unknown	0 (0)	3 (5)
Largest tumour diameter (median)	4·7 cm	4·6 cm
Largest tumour >5 cm		
No	41 (62)	39 (62)
Yes	25 (38)	24 (38)
Prior lines of therapy		
2	35 (53)	33 (52)
3–4	31 (47)	30 (48)
Best response to last prior therapy		
Progressive disease	32 (48)	31 (49)
Stable disease	8 (12)	7 (11)
Partial response	6 (9)	5 (8)
Complete response	6 (9)	8 (13)
Unknown	14 (22)	12 (19)
Cell of origin by Hans algorithm		
Non-GCB	12 (18)	9 (14)
GCB	31 (47)	24 (38)
Unknown	26 (25)	30 (48)
MYC protein ≥40% by IHC		
No	15 (23)	6 (10)
Yes	46 (70)	56 (89)
Unknown	5 (7)	1 (1)
MYC rearrangement		
No	38 (58)	32 (51)
Yes	18 (27)	21 (33)
Unknown	10 (15)	10 (16)
MYC increased copy number		
No	38 (58)	28 (44)
Yes	20 (30)	22 (35)
Unknown	8 (12)	13 (21)

ITT, intention to treat; ECOG, Eastern Cooperative oncology group; LDH, lactate dehydrogenase; GCB, germinal center B; IHC, immunohistochemical staining.

included, consisting of 11 patients from the phase 1 protocol and 52 patients from the phase 2 protocol as depicted in Fig 1. All patients with MYC-altered disease received fimepinostat 60 mg by mouth five days on/2two days off as the starting dose with the exception of four patients enrolled in the phase 1 protocol who received alternate dosing schedules.

Baseline characteristics for the MYC-altered population are described in Table I and are similar to those of the phase 2 ITT population. Response and survival outcomes are described in Table II. For MYC-altered patients the OR rate was 22% (95% CI 13–34%) with seven responding patients each treated by the phase 1 and 2 protocols. Of note, for the subset of patients with double-hit lymphoma (DHL, n = 16; rearrangement of *MYC* and *BCL2* and/or *BCL6*) and double expressor lymphoma (expression of MYC protein ≥40% and BCL2 protein in ≥50% of lymphoma cells by IHC and not also defined as DHL, DEL, n = 28), the OR rates were 19% (95% CI, 4–46%) and 18% (95% CI, 6–37%) respectively. Logistic regression for overall response, performed with all baseline characteristics listed in Table I, revealed that only International Prognostic Index (IPI) score >2 was predictive of response (hazard ratio 0·1, 95% CI, 0·2–0·51, P = 0·006). Of note, 44% of patients who developed progressive disease at any point while on study did so prior to the first allowable date for imaging response assessment per both the phase 1 and 2 protocols (cycle 1, day 15).

Seven out of 14 responding patients remained on treatment for approximately two years or longer (range 22·4–40·5 months), with five patients discontinuing therapy on study while in remission. Additionally, one patient achieving stable disease as best response to treatment remained on therapy for over two years. Clinicopathologic characteristics and outcomes for these patients are listed in Table IV.

In parallel to the phase 1 and 2 clinical trials, VIPER was performed to determine if gene expression patterns correlated with activity of proteins associated with MYC as well as a biomarker pattern of clinical response. For this analysis, 22 pretreatment tumour samples from 11 responding and 11 non-responding patients were included. Significant

Table II. Outcomes for phase 2 intention-to-treat population and phase 1/2 MYC-altered patient populations.

	Phase 2 Group B (n = 46)	Phase 2 All (n = 66)	Phase 1/2 MYC-altered (n = 63)
Overall response	7 (15%, 95% CI 6–29%)	8 (12%, 95% CI 5–22%)	14 (22%, 95% CI 13–34%)
Complete response	4 (9%, 95% CI 2–21%)	5 (8%, 95% CI 3–17%)	8 (13%, 95% CI 6–24%)
Disease control (overall response + stable disease)	15 (33%, 95% CI 20–48%)	20 (30%, 95% CI 20–43%)	31 (49%, 95% CI 36%–62%)
Median progression free survival	1·4 months (95% CI 1·2–1·6 months)	1·4 months (95% CI 1·2–1·5 months)	1·4 months (95% CI 1·3–1·7 months)
Median overall survival	4·2 months (95% CI 2·6–9·1 months)	6·0 months (95% CI 3·8–9·1 months)	6·4 months (95% CI 3·8–13·2 months)
Median duration of response	Not yet reached (95% CI 1·4 months — not yet reached)	Not yet reached (95% CI 1·4 months — not yet reached)	16·5 months (95% CI 2·0 months — not yet reached)
Estimated progression free survival at 6 months	10% (95% CI 3–22%)	9% (95% CI 3–19%)	21% (95% CI 11–32%)
Estimated overall survival at 6 months	41% (95% CI 26–55%)	50% (95% CI 36–61%)	51% (95% CI 37–63%)
Estimated continued response at 6 months	67% (95% CI 20–90%)	71% (95% CI 26–92%)	66% (95% CI 33–86%)

CI, confidence interval.

Table III. Treatment-emergent adverse events (≥10% patients) for phase 2 intention-to-treat population.

Event	Grades 1–2	Grade 3	Grade 4	Grade 5	Total
Diarrhea	36 (54)	12 (18)	0 (0)	0 (0)	48 (72)
Nausea	32 (48)	1 (1)	0 (0)	0 (0)	33 (49)
Thrombocytopenia	9 (13)	15 (22)	3 (4)	0 (0)	27 (40)
Fatigue	24 (36)	0 (0)	0 (0)	0 (0)	24 (36)
Decreased appetite	22 (33)	0 (0)	0 (0)	0 (0)	22 (33)
Hypokalemia	14 (21)	8 (12)	0 (0)	0 (0)	22 (33)
Vomiting	18 (27)	1 (1)	0 (0)	0 (0)	19 (28)
Anemia	7 (10)	11 (16)	0 (0)	0 (0)	18 (27)
Constipation	14 (21)	0 (0)	0 (0)	0 (0)	14 (21)
Neutropenia	2 (3)	9 (13)	3 (4)	0 (0)	14 (21)
Pyrexia	13 (19)	0 (0)	0 (0)	0 (0)	13 (19)
Hypomagnesemia	11 (16)	1 (1)	0 (0)	0 (0)	12 (18)
Abdominal pain	8 (12)	3 (4)	0 (0)	0 (0)	11 (16)
Dizziness	11 (16)	0 (0)	0 (0)	0 (0)	11 (16)
Dyspnea	9 (13)	2 (3)	0 (0)	0 (0)	11 (16)
White blood cell count decreased	5 (7)	4 (6)	1 (1)	0 (0)	10 (15)
Acute kidney injury	6 (9)	3 (4)	0 (0)	0 (0)	9 (13)
Arthralgia	9 (13)	0 (0)	0 (0)	0 (0)	9 (13)
Cough	9 (13)	0 (0)	0 (0)	0 (0)	9 (13)
Lymphocyte count decreased	3 (4)	4 (6)	2 (3)	0 (0)	9 (13)
Pain in extremity	7 (10)	2 (3)	0 (0)	0 (0)	9 (13)
Peripheral edema	8 (12)	0 (0)	0 (0)	0 (0)	8 (12)
Weight decreased	7 (10)	1 (1)	0 (0)	0 (0)	8 (12)
Dehydration	4 (6)	3 (4)	0 (0)	0 (0)	7 (10)
Hypophosphataemia	2 (3)	5 (7)	0 (0)	0 (0)	7 (10)

enrichment of 67 B-cell context-specific MYC-interacting proteins[30] was observed among the proteins most differentially active between fimepinostat responder and non-responders [$P < 0{\cdot}001$, Gene Set Enrichment Analysis (GSEA)]. As part of a the OncoMarker biomarker discovery algorithm,[25] a Neural-Network classifier was trained on protein activity profiles of analysed tumour samples. The analysis identified three proteins—PBXIP1, ETS1 and ANGPTL3—as Master Regulators (MRs) of fimepinostat sensitivity (Fig 2A and Table SI), yielding optimal predictive power based on leave-one-out cross-validation (LOO-CV) [Area Under Receiver Operating Characteristic Curve (AUC) = 0·901, 95% CI 0·776–1 (Fig 2B)]. The biomarker correctly identified 9 of 11 responding (82%) and misclassified only 2 of 11 non-responding (18%) patients (Fig 2A). When restricting this analysis to 16 MYC-altered patients, the fimepinostat-sensitivity biomarker had equivalent performance [LOO-CV AUC = 0·921, 95% CI 0·789–1 (Fig 2C)] and correctly identified 8 of 9 responding (89%) and misclassified only 1 of 7 non-responding (14%) patients (Fig 2A).

Discussion

While a modest OR rate and median progression-free survival was experienced by patients with multiply R/R DLBCL/HGBL treated with dual HDAC/PI3K inhibitor fimepinostat in the phase 2 setting, an exploratory pooled analysis of patients with MYC-altered disease treated on this trial as well as the preceding phase 1 study revealed an OR rate of 22% with a median duration of response of 16·5 months and 66% of responding patients were estimated to have a continued response at six months. Furthermore, seven responding patients remained on treatment for approximately two years without disease progression. Of note, there were only three responding patients treated on the phase 1 and 2 studies who were not classified as having MYC-altered disease, and only one remained on treatment for a similarly long duration.

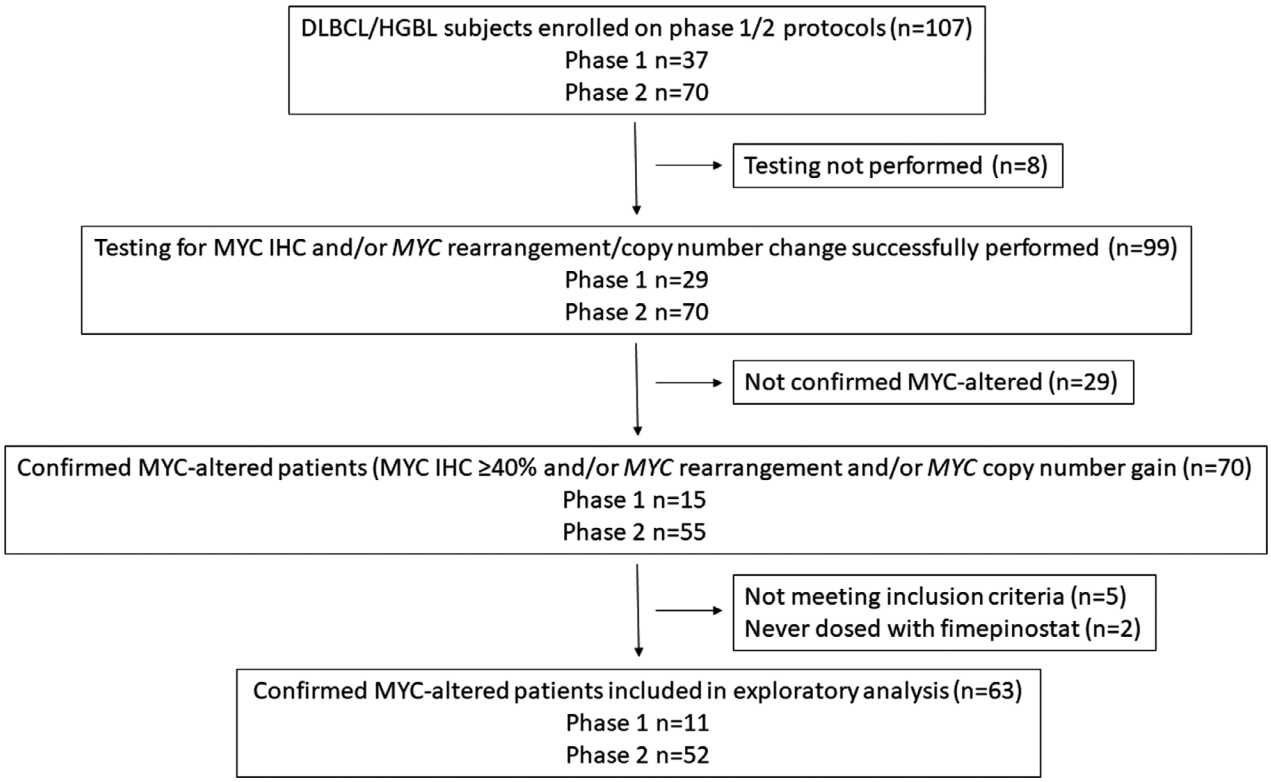

Fig 1. Patient selection for exploratory analysis of patients with MYC-altered disease.

The mechanism of action and preclinical activity of fimepinostat *in vitro* and *in vivo*,[15] in combination with the clinical observations that nearly all patients responding to fimepinostat treated on the phase 2 study harboured MYC-altered disease as well as the large proportion of prolonged responses in responding patients with MYC-altered disease treated on both the phase 1 and 2 studies, support consideration of MYC alteration, a known prognostic marker in DLBCL/HGBL, as a predictive biomarker for response to fimepinostat in patients with multiply R/R DLBCL/HGBL and plans for further investigation of fimepinostat in those patients with MYC-altered disease. Consideration of key findings from our pooled exploratory analysis of these patients may help to optimize future clinical trial design.

It is notable that approximately half of all patients with MYC-altered disease included in the exploratory pooled analysis who ultimately developed progressive disease did so prior to the time of the first planned imaging assessment. Multiply R/R DLBCL/HGBL with MYC alterations can grow rapidly, and it is possible that disease progression may occur in these patients prior to the minimum duration of exposure to fimepinostat required to realize efficacy. While combinations of small-molecule inhibitors with immunochemotherapy regimens for fixed durations have been previously studied in patients with R/R DLBCL,[31] a more relevant clinical trial design may be to initially combine a small-molecule inhibitor with immunochemotherapy for a fixed duration and/or until objective response is achieved followed by maintenance fimepinostat monotherapy, a strategy which is being pursued with another small-molecule inhibitor in this clinical setting (NCT04442022). However, given the overlapping toxicities of fimepinostat with cytotoxic chemotherapy, a rapidly acting non-cytotoxic agent such as polatuzumab vedotin[32] may be a better approach in combination with fimepinostat. Utilizing a rapidly acting agent as a "bridge" to allow patients to achieve optimal exposure duration to fimepinostat in order to respond has the potential to overcome early treatment failure and allow a greater proportion of patients treated with fimepinostat to experience prolonged durations of response to subsequent treatment with monotherapy.

It is also revealing that a biomarker classification not known to be prognostic in DLBCL/HGBL derived by VIPER analysis may predict for response to fimepinostat in patients with MYC-altered disease, only misclassifying one responding and one non-responding patient whose tumours were analysed. The use of VIPER has led to identification of a similar predictive biomarker when applied to patients treated with another small-molecule inhibitor in this clinical setting.[33] Validation of this biomarker identified in MYC-altered patients treated with fimepinostat should be pursued in future clinical trials in order to determine if biomarker-guided selection can be validated as a feasible strategy for offering this therapy to patients.

Table IV. Selected clinicopathologic features and outcomes for phase 1/2 MYC-altered patients with prolonged duration on therapy.

ID	Age	Sex	Study	Stage	LDH elevated	IPI score >2	MYC status	Largest tumour diameter (mm)	Number of prior lines of therapy	Last prior treatment best overall response	Prior stem cell transplant	Fimepinostat best overall response	Time to first response (months)	Progression free suvival (months)	Duration of response (months)	Reason for discontinuation	Subsequent therapy	Overall survival (months)	Alive at last follow-up
1	46	M	Ph 1	III	No	No	IHC	46	2	SD	No	CR	4·0	40·5	36·5	End of study	Fimepinostat (CU)	41·3	Yes
2	73	F	Ph 1	Unk	No	Unk	CN	34	3	Unk	Yes	CR	1·2	39·4	38·2	End of study	Unk	39·4	Yes
3	69	M	Ph 2	IV	Yes	No	IHC (DEL)	49	3	PR	No	CR	2·7	30·0	27·3	End of study	Fimepinostat (CU)	32·9	Yes
4	49	F	Ph 1	IV	No	No	IHC, RA	40	4	PD	No	PR	11·2	27·7	16·5	PD	Unk	28·2	Yes
5	38	M	Ph 1	III	No	No	IHC (DEL)	27	3	PD	No	SD		25·7		Investigator decision	Active observation	28·4	Yes
6	53	M	Ph 2	IV	Yes	Yes	IHC, RA (DHL)	52	2	PD	No	PR	2·8	24·4	21·7	End of study	CART19	27·9	Yes
7	54	F	Ph 2	II	Yes	No	IHC, RA (DHL)	19	2	PD	No	CR	2·8	23·8	21·0	End of study	CART19	27·2	Yes
8	71	M	Ph 1	III	No	No	IHC, CN	32	3	CR	Yes	CR	7·7	22·4	14·7	PD	Unk	22·4	Yes

M, male; F, female; Ph, phase; LDH, lactate dehydrogenase; Unk, Unknown; IPI, International Prognostic Index; IHC, immunohistochemical staining; CN, copy number increase; DEL, double expressor lymphoma; RA, rearrangement; DHL, double-hit lymphoma; DEL, double expressor lymphoma; SD, stable disease; PR, partial response; PD, progressive disease; CR, complete response; CU, compassionate use; CART19, chimaeric antigen receptor-modified T cells directed against CD19.

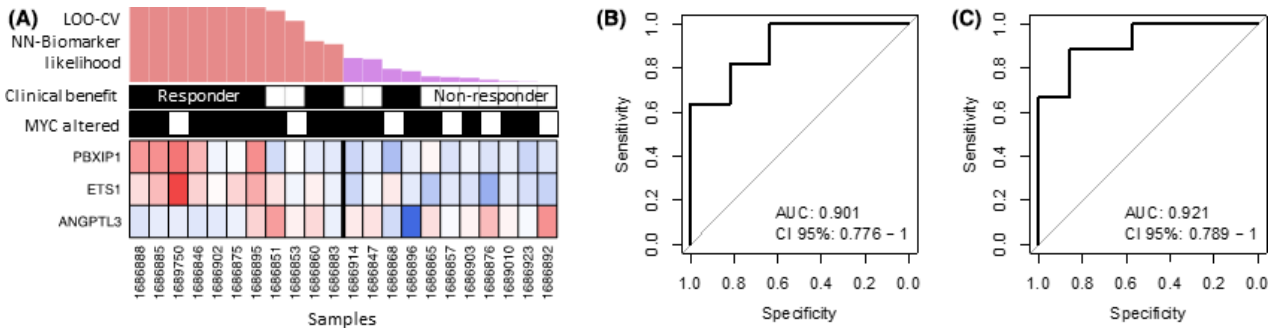

Fig 2. Leave-one-outcross-validation (LOO-CV) analysis for fimepinostat response biomarkers. (A) Heatmap showing the Virtual Inference of Protein-activity by Enriched Regulon analysis (VIPER)-inferred activity for the three fimepinostat response Master Regulator (MR) proteins used by the biomarkers (rows) for all samples. The clinical samples included in the analysis (columns) were rank-sorted based on the predicted likelihood of response by the NN-biomarker (barplot above the heatmap), estimated using LOO-CV. Patients that responded to fimepinostat [complete response (CR) and partial response (PR)] and patients that did not respond for fimepinostat [progressive disease (PD)] are shown in black and white, respectively (Clinical benefit row). Patients with MYC-altered disease are indicated in black in the MYC-altered row. (B, C) receiver operating characteristic (ROC) analysis for the LOO-CV performed on all samples (n = 22; B) and only on the MYC-altered samples (n = 16; C). Area under the ROC curve (AUC) and its 95% confidence interval (CI) is shown inside each plot.

The strength of our analysis includes reporting of the largest known cohort of patients with multiply R/R DLBCL/HGBL with MYC-altered disease who were treated single-agent therapy prospectively on clinical trials, which is relevant given available preclinical data which demonstrate that HDAC and PI3K inhibition down-regulate activity of MYC. Additionally, given that DLBCL/HGBL with MYC alterations is both associated with a poor prognosis when treated with standard therapies and is likely enriched for in those patients with multiply R/R DLBCL/HGBL, this report of well-tolerated oral agent with clinical activity in this patient population is of interest to the lymphoma community. The weaknesses of our analysis include a lack of testing for MYC alterations in all patients treated in the phase 1 and 2 studies, as well as the small sample size of patients without MYC-altered disease, which may affect the validity of comparative statistical analyses based upon MYC alteration status.

In conclusion, objective responses were observed in multiply R/R DLBCL/HGBL patients treated with fimepinostat monotherapy in the phase 2 setting, and an exploratory analysis of patients with MYC-altered disease treated with fimepinostat in both the phase 1 and 2 setting revealed a higher proportion of responses with prolonged durations of response in this cohort. These findings support further investigation of fimepinostat in patients with MYC-altered DLBCL/HGBL, with consideration of combination-based therapies and additional exploration of predictive biomarkers, in hopes of allowing a greater proportion of patients to derive clinical benefit from treatment with this agent.

Funding information

Research funding provided by Curis, Inc.

Author contributions

DJL treated subjects, analysed data, wrote the paper. SKB, RR, CB, SI, KK, INM, SMS, DAS andYO treated subjects, critically revised the paper. MA, AC, YS and GB performed research, contributed essential reagents or tools, analysed data, critically revised the paper. JP, EM, RVR and RM designed the study, performed research, analysed data, critically revised the paper. All authors approved the submitted version of the paper.

Conflicts of interest

DJL: research funding: Takeda, Curis, Triphase. Consulting/Advisory boards: Morphosys, Karyopharm, Celgene. SKB: Honorarium: Pfizer, Acrotech, Janssen, Seattle Genetics, Atara. Research funding: Seattle Genetics. Consultancy: Monsanto. RR: Consultancy: Seattle Genetics, Sandoz-Novartis, Pharmacyclics, Jannsen, Bristol-Myers Squibb. CB: research funding: Janssen, Novartis, Epizyme, Xynomics, Bayer, Autolus, Roche. Consulting/advisory boards: Life Sci, GLG, Juno/Celgene, Seattle Genetics, Kite, Karyopharm, TG Therapeutics. Honorarium: Dava Oncology. SI: research grant and consultant: Seattle Genetics, Rhizen, Daiichi Sankyo, Trillium; research grant- Merck, Affimed, Spectrum. KK: honoraria: Celgene, Janssen, Takeda, Novartis, Ascentage Pharma, Applied Clinical Intelligence LLC, Epizyme, Inc., Pharmacyclics, Inc., Karyopharm, SEATTLE GENETICS, AstraZeneca, Denovo Biopharma, LLC, SANOFI-AVENTIS. INM: none. SMS: research funding: Acerta, Karyopharm, FortySeven, Genentech/Roche, TG Therapeutics, Celgene, Pharmacyclics. Consultancy: Jannsen, BMS, Karyopharm, Genetech/Roche, TG Therapeutics, Celgene. DAS: consulting/advisory boards: Amgen, MorphoSys. MA: employment and equity

holder: DarwinHealth, Inc. AC: employment and equity holder: DarwinHealth, Inc. YS: employment and equity holder: DarwinHealth, Inc. GB: employment and equity holder: DarwinHealth, Inc. JP: employment: Curis, Inc. RS: employment: Curis, Inc. EM: employment: Curis, Inc. RVR: employment: Curis, Inc. REM: employment: Curis, Inc. YO: employment: Genetech.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. VIPER-inferred protein activity.

References

1. Dang CV, O'Donnell KA, Zeller KI, Nguyen T, Osthus RC, Li F. The c-Myc target gene network. *Semin Cancer Biol*. 2006;**16**:253–64.
2. Meyer N, Penn LZ. Reflecting on 25 years with MYC. *Nat Rev Cancer*. 2008;**8**:976–90.
3. Barrans S, Crouch S, Smith A, Turner K, Owen R, Patmore R, et al. Rearrangement of MYC is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. *J Clin Oncol*. 2010;**28**:3360–5.
4. Savage KJ, Johnson NA, Ben-Neriah S, Connors JM, Sehn LH, Farinha P, et al. MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy. *Blood*. 2009;**114**:3533–7.
5. Cuccuini W, Briere J, Mounier N, Voelker HU, Rosenwald A, Sundstrom C, et al. MYC+ diffuse large B-cell lymphoma is not salvaged by classical R-ICE or R-DHAP followed by BEAM plus autologous stem cell transplantation. *Blood*. 2012;**119**:4619–24.
6. Li S, Seegmiller AC, Lin P, Wang XJ, Miranda RN, Bhagavathi S, et al. B-cell lymphomas with concurrent MYC and BCL2 abnormalities other than translocations behave similarly to MYC/BCL2 double-hit lymphomas. *Mod Pathol*. 2015;**28**:208–17.
7. Valera A, Lopez-Guillermo A, Cardesa-Salzmann T, Climent F, Gonzalez-Barca E, Mercadal S, et al. MYC protein expression and genetic alterations have prognostic impact in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. *Haematologica*. 2013;**98**:1554–62.
8. Meriranta L, Pasanen A, Alkodsi A, Haukka J, Karjalainen-Lindsberg ML, Leppa S. Molecular background delineates outcome of double protein expressor diffuse large B-cell lymphoma. *Blood Adv*. 2020;**4**:3742–53.
9. Landsburg DJ, Nasta S, Svoboda J, Mato AR, Schuster SJ, Sargent RL. Outcomes of patients with MYC-expressing aggressive B cell non-Hodgkin lymphomas who fail two or more lines of therapy. *J Clin Oncol*. 2018;**36**:e19528.
10. Landsburg DJ. Management of patients with MYC-altered lymphomas. *Curr Hematol Malig Rep*. 2016;**11**:208–17.
11. Assouline SE, Nielsen TH, Yu S, Alcaide M, Chong L, MacDonald D, et al. Phase 2 study of panobinostat with or without rituximab in relapsed diffuse large B-cell lymphoma. *Blood*. 2016;**128**:185–94.
12. Batlevi CL, Crump M, Andreadis C, Rizzieri D, Assouline SE, Fox S, et al. A phase 2 study of mocetinostat, a histone deacetylase inhibitor, in relapsed or refractory lymphoma. *Br J Haematol*. 2017;**178**:434–41.
13. Flinn IW, O'Brien S, Kahl B, Patel M, Oki Y, Foss FF, et al. Duvelisib, a novel oral dual inhibitor of PI3K-delta, gamma, is clinically active in advanced hematologic malignancies. *Blood*. 2018;**131**:877–87.
14. Lenz G, Hawkes E, Verhoef G, Haioun C, Thye Lim S, Seog Heo D, et al. Single-agent activity of phosphatidylinositol 3-kinase inhibition with copanlisib in patients with molecularly defined relapsed or refractory diffuse large B-cell lymphoma. *Leukemia*. 2020;**34**:2184–97.
15. Sun K, Atoyan R, Borek MA, Dellarocca S, Samson ME, Ma AW, et al. Dual HDAC and PI3K inhibitor CUDC-907 downregulates MYC and suppresses growth of MYC-dependent cancers. *Mol Cancer Ther*. 2017;**16**:285–99.
16. Pei Y, Liu KW, Wang J, Garancher A, Tao R, Esparza LA, et al. HDAC and PI3K antagonists cooperate to inhibit growth of MYC-driven medulloblastoma. *Cancer Cell*. 2016;**29**:311–23.
17. Bouchard C, Marquardt J, Bras A, Medema RH, Eilers M. Myc-induced proliferation and transformation require Akt-mediated phosphorylation of FoxO proteins. *EMBO J*. 2004;**23**:2830–40.
18. Bouchard C, Lee S, Paulus-Hock V, Loddenkemper C, Eilers M, Schmitt CA. FoxO transcription factors suppress Myc-driven lymphomagenesis via direct activation of Arf. *Genes Dev*. 2007;**21**:2775–87.
19. Younes A, Berdeja JG, Patel MR, Flinn I, Gerecitano JF, Neelapu SS, et al. Safety, tolerability, and preliminary activity of CUDC-907, a first-in-class, oral, dual inhibitor of HDAC and PI3K, in patients with relapsed or refractory lymphoma or multiple myeloma: an open-label, dose-escalation, phase 1 trial. *Lancet Oncol*. 2016;**17**:622–31.
20. Oki Y, Kelly KR, Flinn I, Patel MR, Gharavi R, Ma A, et al. CUDC-907 in relapsed/refractory diffuse large B-cell lymphoma, including patients with MYC-alterations: results from an expanded phase I trial. *Haematologica*. 2017;**102**:1923–30.
21. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;**103**:275–82.
22. Cheson BD, Pfistner B, Juweid ME, Gascoyne RD, Specht L, Horning SJ, et al. Revised response criteria for malignant lymphoma. *J Clin Oncol*. 2007;**25**:579–86.
23. Alvarez MJ, Shen Y, Giorgi F, Lachmann A, Ding BB, Ye BH, et al. Functional characterization of somatic mutations in cancer using network-based inference of protein activity. *Nat Genet*. 2016;**48**:838–47.
24. Neal M. Assay Validation Review, Wadsworth Center, NY State Department of Health, PFI: 7313, Project ID: 63859. 2019.
25. Chari A, Vogl DT, Gavriatopoulou M, Nooka AK, Yee AJ, Huff CA, et al. Oral selinexor-dexamethasone for triple-class refractory multiple myeloma. *N Engl J Med*. 2019;**381**:727–38.
26. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet*. 2000;**25**:25–9.
27. Hongxu DH, Douglass EF, Sonabend AM, Mela A, Bose A, Gonzalez C, et al. Quantitative assessment of protein activity in orphan tissues and single cells using the metaVIPER Algorithm. *Nat Methods*. 2018;**9**:1471.
28. Basso K, Margolin AA, Stolovitzky G, Klein U, Dalla-Favera R, Califano A. Reverse engineering of regulatory networks in human B cells. *Nat Genet*. 2005;**37**:382–90.
29. Bishop CM. Neural networks for pattern recognition. Oxford: Clarendon Press; 1995.
30. Lefebvre C, Rajbhandari P, Alvarez MJ, Bandaru P, Lim WK, Sato M, et al. A human B-cell interactome identifies MYB and FOXM1 as master regulators of proliferation in germinal centers. *Mol Syst Biol*. 2010;**6**:377.
31. Rhodes J, Landsburg DJ. Small-molecule inhibitors for the treatment of diffuse large B cell lymphoma. *Curr Hematol Malig Rep*. 2018;**13**:356–68.
32. Hughes ME, Nasta SD, Gerson JN, Svoboda J, Chong EA, Schuster SJ, et al. Time-to-response for patients with relapsed/refractory aggressive B cell non-Hodgkin lymphoma treated with polatuzumab-based therapy. *Hematol Oncol*. 2021;**39**:420.
33. Walker CJ, Shen Y, Alvarez M, Chang H, Shah J, Shacham S, et al. A six-protein activity signature defines favorable response to selinexor treatment for patients with diffuse large B-cell lymphoma (DLBCL). *Blood*. 2020;**136**:31–2.