Preliminary evidence of a molecular predictor of tazemetostat response, beyond EZH2 mutation, in NHL patients via characterization of archive tumor and circulating tumor DNA

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INTRODUCTION

- B-cell malignancies may depend on enhancer of zeste homolog 2 (EZH2; a histone methyl transferase) to perpetuate a less differentiated state, with activating mutations of EZH2 being potential oncogenic drivers
- Tazemetostat is an oral, first-in-class, potent, and highly selective inhibitor of EZH2 currently in phase 2 clinical development for the treatment of relapsed or refractory non-Hodgkin lymphoma (NHL)
- In the phase 1 part of a phase 1/2 study (NCT01897571) of adult patients with advanced solid tumors or B-cell lymphomas, objective responses to tazemetostat were observed in patients with tumors exhibiting mutated or wild-type EZH2
- An ongoing phase 2 study (NCT01897571) is enrolling patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) or follicular lymphoma (FL) to assess the efficacy and safety of tazemetostat (results reported separately)
  - Patients with tumors exhibiting mutated or wild-type EZH2 are eligible to participate
  - The primary study endpoint is objective overall response rate
- Here, we report the molecular analysis of tumor material obtained in the phase 2 study and its associations with preliminary response data, including the discovery of a novel candidate molecular predictor of tazemetostat response

METHODS

- During screening, archive tumor- and/or plasma-derived circulating tumor DNA (ctDNA) samples were obtained
- Archive tumor was analyzed prospectively for EZH2-activating mutations Y646F, Y646N, Y646X (Y646H, Y646C, and Y646S), A682G, and A692V using a **cobas®** EZH2 Mutation Test (Roche Molecular Systems, Inc., Pleasanton, CA, USA; in development)
- Next generation sequencing (NGS) was performed retrospectively on DNA derived from archive tumor and plasma to identify somatic mutations, amplifications, and translocations using the Epizyme NHL-specific 62-gene panel[1] on the Illumina HiSeq 2500 platform (Illumina, Inc., San Diego, CA, USA) with 100 bp paired-end reads (Table 1)
  - Average target coverage for the tissue panel was 1250 X, while coverage for the ctDNA was approximately 20,000 X for mutations and 3700 X for structural alterations

Table 1. Content for a targeted NGS panel representing 62 genes reported to be mutated in >5% DLBCL or FL patients

Custom Lymphoma CancerSelect-R Sequence – Mutation Gene List			
Gene Name		**Gene Name**	
PRDM1[a]	CD58	KRAS	PTPRD
EZH2[a]	CD798	MEF2B	RB1
KDM6A[a]	CDKN2A	MYC	S1PR2
KMT2D[a]	CREBBP	MYD88	SGK1
ARID1A	EP300	NOTCH1	SMARCB1
ATM	FOXO1	NOTCH2	SOCS1
B2M	GNA13	NRAS	STAT6
BCL2	HIST1H1B	PIK3CA	TBL1XR1
BCL6	HIST1H1C	PIM1	TNFAIP3
BCL7A	HIST1H1E	POU2F2	TNFRSF14
BRAF	IKZF3	PTEN	TP53
BTG1	IRF4	PTPN1	XPO1
CARD11	ITPKB	PTPN11	
CCND3	KIT	PTPN6	

Custom Lymphoma CancerSelect-R Translocation Gene List		Custom Lymphoma CancerSelect-R Amplification Gene List
Gene Name	**Sequence Region(s) Included**	**Gene Name**
ALK	ALK_NM_004304_Intron19	BCL2
BCL2	BCL2_MCR_Breakpoint_Region	CD274 (PDL1)
BCL2	BCL2_MBR_Breakpoint_Region	PDCD1LG2 (PDL2)
BCL6	Entire Gene	FOXP1
CIITA	Entire Gene	JAK2
MYC	Entire Gene + 40kBP upstream	KDM4c
CD274 (PDL1)	Entire Gene	REL
PDCD1LG2 (PDL2)	Entire Gene	

[a]Full coding sequence analyzed. Specific exons were otherwise sequenced for all genes in the mutation list.

- Sequencing data were aligned to the human reference sequence (hg19) and analyzed using validated cancer genome analysis algorithms (Personal Genome Diagnostics, Baltimore, MD, USA)
- The data cutoff was June 01, 2017

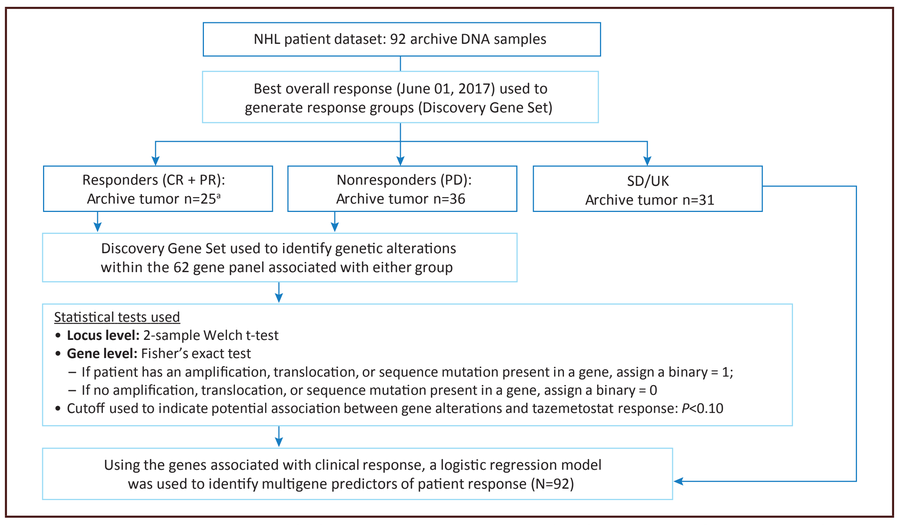
Table 2. Demographics and disease characteristics for phase 2 patient cohort and subset sequenced by NGS

Characteristic	Phase 2 Total Cohort	NGS Cohort
n	204	92
Age, median years	66	65
Males, %	58	63
ECOG PS, median (range)	1 (0–2)	0 (0–2)
Prior lines of therapy, n (%)		
1–2	61 (30)	26 (28)
3–4	83 (41)	35 (38)
≥ 5	60 (29)	31 (34)
median	3	4
Median time from initial diagnosis, years	2.7	3.5
Median time from last prior therapy, weeks	15.1	13.9
Refractory to last regimen, n (%)	122 (60)	52 (57)
DLBCL, n (%)		
EZH2 WT[a]	120 (59)	57 (62)
EZH2 mutant[a]	17 (8)	6 (6)
FL, n (%)		
EZH2 WT[a]	54 (26)	24 (26)
EZH2 Mutant[a]	13 (6)	5 (5)

[a]Determined by **cobas®** EZH2 Mutation Test.
ECOG PS, Eastern Cooperative Oncology Group performance status; WT, wild-type.

- EZH2 mutation detection rates in archive tumor were ~12% in DLBCL and 19% in FL in the total phase 2 cohort (Table 2), consistent with previous reports

Figure 1. Analytical and statistical methods

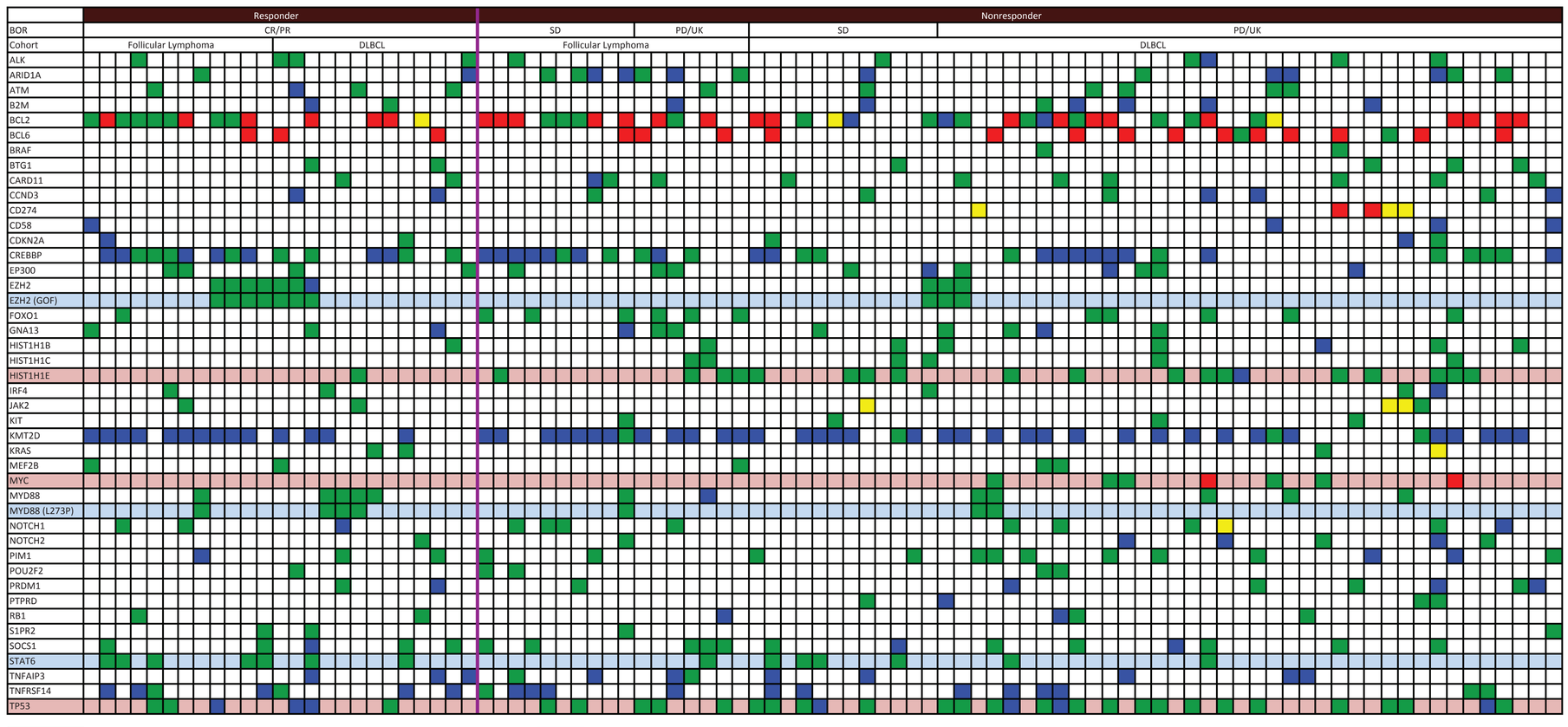


[a]Ten patients had a complete response (CR) and 15 had a partial response (PR) (total number of responders=25).

NHL, non-Hodgkin lymphoma; SD, stable disease; PD, progressive disease; UK, unknown response.

RESULTS

Figure 2. Somatic mutations, amplifications, and translocations detected in 92 NHL patients by best overall response



- Mutations in EZH2, STAT6, or MYD88 in wild-type EZH2 patients were associated with response (*P*<0.1), whereas mutations in HIST1H1E, TP53, or MYC (*P*<0.08) were associated with nonresponse (Table 4)
  - Activating mutations in EZH2 and MYD88 were mutually exclusive

Table 4. Variants identified as significantly associated with tazemetostat response using gene-level analysis of archive tumor tissue (responders n=25; nonresponders n=36)

Gene	TP53	HIST1H1E	EZH2	MYC	STAT6	MYD88 L273P	All Patients
Responder, n	6	1	7	0	7	4	25
Nonresponder, n	21	14	2	7	3	2	36
P-value	0.0079	0.02	0.0253	0.0727	0.0753	0.0841	NA
Response preference	WT	WT	Mutant	WT	Mutant	Mutant	NA

Activating mutations in EZH2 and MYD88 are mutually exclusive.

- Patients matching a multigene predictor consisting of wild-type MYC and/or HIST1H1E but with mutated STAT6 and/or MYD88 in archive tumor had an objective overall response rate of ~58% (11/19), whereas patients who did not match this profile had an objective overall response rate of ~19% (14/73), indicating potential for these four genes to predict response to tazemetostat (Table 5)

Table 5. Results of predictive modelling to identify potential multigene predictors of response to archival tumor

Multigene Predictor Performance for Archival Tumor Response from NHL Patients		
	Patients Matching Profile n (%)	**Patients Not Matching Profile n (%)**
Genetic Profile 1	19	73
Responder (CR/PR)	11 (57.9)	14 (19.2)
Nonresponder (SD/PD/UK)	8 (42.1)	59 (80.8)
Genetic Profile 2	26	66
Responder (CR/PR)	15 (57.7)	10 (15.2)
Nonresponder (SD/PD/UK)	11 (42.3)	56 (84.8)

Genetic Profile 1 = MYC wt + HIST1H1E wt + (STAT6/any or MYD88/L273).
Genetic Profile 2 = MYC wt + HIST1H1E wt + (STAT6/any or MYD88/L273 or EZH2/Y646, A682, A692).

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Please contact Stephen Blakemore (sblakemore@epizyme.com) with questions and comments.

Reference
1. Daigle SR, et al. Poster presented at the American Association for Cancer Research Annual Meeting, 2016. Poster #137.

RESULTS (CONTINUED)

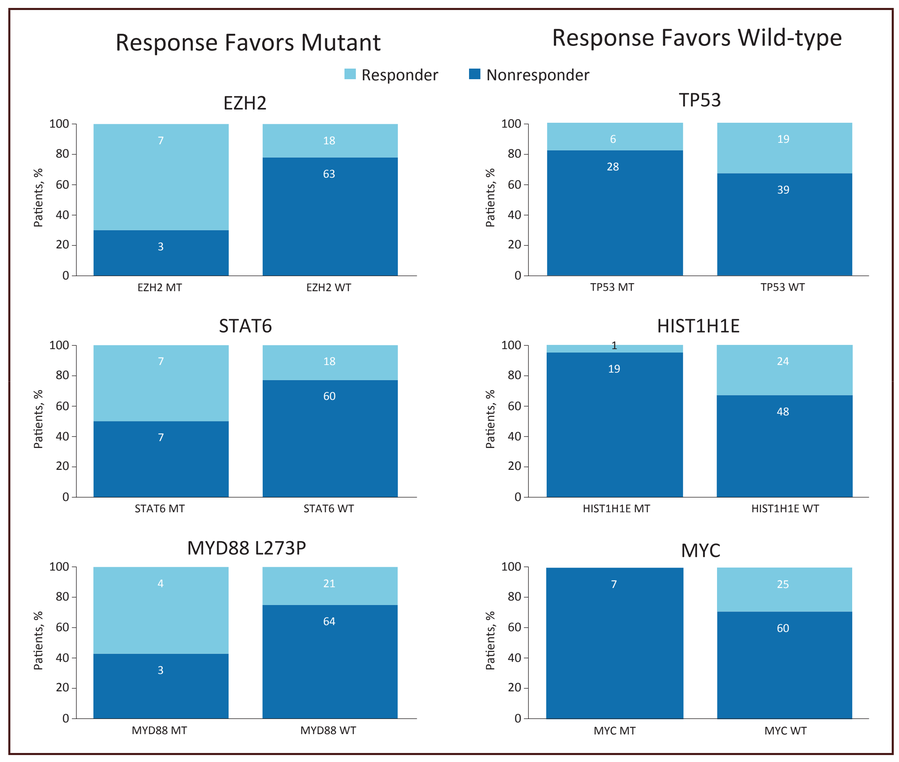
- Regardless of the technology or sample type used, the concordance rate for detection of EZH2 status was >95%
  - NGS and **cobas®** Test of archive tumor samples was 98.9% concordant (n=92) with 11 EZH2 activating mutation cases detected (Table 3)
  - Concordance of EZH2 status between archive tumor and ctDNA samples was 97% (n=125)

Table 3. Comparison of EZH2 activating mutation detection between cobas® EZH2 Mutation Test and NGS analysis in archive tumor and ctDNA

Cohort Designation	Cell of Origin (Nanostring)	EZH2 cobas® Test	Tumor Content for cobas® Test (%)	Archive Tumor NGS Result (vaf) (%)	ctDNA NGS Result (vaf)	Clonal or Subclonal EZH2 Mutation[a]
GCB-DLBCL EZH2 MT	GCB DLBCL	Y646F	100	EZH2 Y646F (10)	EZH2 Y646F (1.3%)	Subclonal
Non-GCB DLBCL	GCB DLBCL	Y646X	20	EZH2 Y646H (19)	EZH2 Y646H (12.7%)	Clonal
Non-GCB DLBCL	Unclassified	Y646F	50	EZH2 Y646F (23)	Data pending	Clonal
GCB-DLBCL EZH2 MT	GCB DLBCL	Y646F	100	EZH2 Y646F (38)	EZH2 Y646F (8.9%)	Clonal
FL EZH2 mutant	N/A	Y646F	100	EZH2 Y646F (8)	Not detected	Subclonal
GCB-DLBCL EZH2 MT	Not performed	A682G	95	EZH2 A682G (34)	EZH2 A682G (0.9%)	Clonal
FL EZH2 mutant	N/A	Y646N	90	EZH2 Y646N (22)	Low DNA yield	Clonal
FL EZH2 mutant	N/A	Y646X	100	EZH2 Y646S (22)	EZH2 Y646S (6.6%)	Clonal
GCB-DLBCL EZH2 MT	GCB DLBCL	Y646X	UK	EZH2 Y646H (25)	EZH2 Y646H (28%)	Clonal
FL EZH2 mutant	N/A	Y646X	100	EZH2 Y646H (2)	EZH2 Y646H (0.7%)	Subclonal
FL EZH2 mutant	N/A	Y646X	100	Not detected	Not detected	N/A

[a]Patients determined to have EZH2 mutant (MT) tumor DNA copies ≥20% were considered clonal.

Figure 3. Variants identified as significantly associated with tazemetostat response using gene level analysis of archive tumor tissue distributed by response-favoring mutant and response-favoring wild-type gene (N=92)



CONCLUSIONS

- Molecular genetic profiling of NHL patients identified potential predictors of response to tazemetostat beyond EZH2-activating mutations and offered new insights into mechanisms of response in EZH2 wild-type patients
- Plasma-based ctDNA screening may be a viable method to identify NHL patients with EZH2-activating mutations in the absence of archive tumor samples
- The phase 2 study and additional biomarker analyses are ongoing