blood advances

TO THE EDITOR:

Genetic characterization and drug sensitivity study of newly derived HGBL double/triple-hit lymphoma cell lines

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As a poor-risk group of aggressive B-cell lymphomas (BCLs), high-grade BCLs harboring *MYC* and *BCL2* and/or *BCL6* rearrangements high grade B-cell lymphoma-double/triple-hit (HGBL-D/TH) are currently a subject of intense clinical and research interest. New drugs, particularly targeted therapeutic agents, are increasingly being developed and entering the clinic in recent years.[1] A panel of well-characterized HGBL-D/TH cell lines will be valuable for preclinical drug screening and development. There are so far few HGBL-D/TH cell lines that have comprehensive genomic data.[2-4] Here, we reported 2 new double/triple-hit lymphoma (D/THL) cell lines named "COH-DHL1" and "COH-THL1" with comprehensive characterization.

COH-DHL1 and COH-THL1 were derived from 2 male patients at City of Hope, and both were Epstein-Barr virus (EBV)-negative, as shown by the Epstein-Barr nuclear antigen polymerase chain reaction assay (Figure 1A). Their doubling times were 47.8 hours and 27 hours, respectively (Figure 1B). When we injected 10 million cells of each cell line subcutaneously into NSG mice, all 3 replicates developed tumors within 3 weeks (Figure 1C). Fingerprinting showed that the 2 cell lines were unique without any identical cell lines in the Cellosaurus database (supplemental Figure 1 in the data supplement). Flow cytometry showed consistent immunophenotype between the 2 cell lines, their 1- and 2-month cultures, and their mice tumors in terms of CD45, CD19/CD20, and immunoglobulin light chains (supplemental Figure 2 and Figure 1D). For COH-THL1, whose original tumor was available, fingerprinting and flow cytometry also showed consistency with its origin (supplemental Figures 1B and 2B). All animal procedures in the mice experiment were in accordance with the guidelines and approved by the Administrative Panel on Laboratory Animal Care at City of Hope Comprehensive Cancer Center.

The 2 cell lines were further characterized with 2 other reported D/THL cell lines (DOGKIT[5] and CS-THL1[6]) for comparison. We tested the sensitivity of the 4 cell lines along with Jijoye and D/THL cell line (SU-DHL6) to 10 drugs targeting BCL2, MYC, BTK, PI3K, or CDK (Figure 1E). BCL2 is an important antiapoptotic protein that synergizes with other oncogenes, such as MYC, to promote lymphoma development and could be a critical factor for HGBL-D/TH survival. Inhibiting BCL2 by potent BH3 mimetics such as ABT-199 demonstrated a remarkable clinical response in selected patients.[7] This is also demonstrated by the higher sensitivity of the 5 HGBL-D/TH cell lines to ABT199, compared with Jijoye, which is a Burkitt lymphoma (BL) lacking BCL2 expression.

MYC, as a key oncogene,[8] likely plays a crucial role in HGBL-D/TH, making it an excellent therapeutic target. Although it is yet impossible to directly inhibit MYC function, many strategies have been employed to indirectly inhibit MYC activity. In this study, we attempted to: (1) impair *MYC* transcription by inhibiting the BRD4 (bromodomain-containing 4) using JQ-1[9]; (2) interfere with *MYC* mRNA translation using the eukaryotic initiation factor (eIF) 4A inhibitor silvestrol[10]; (3) reduce MYC protein stability using

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

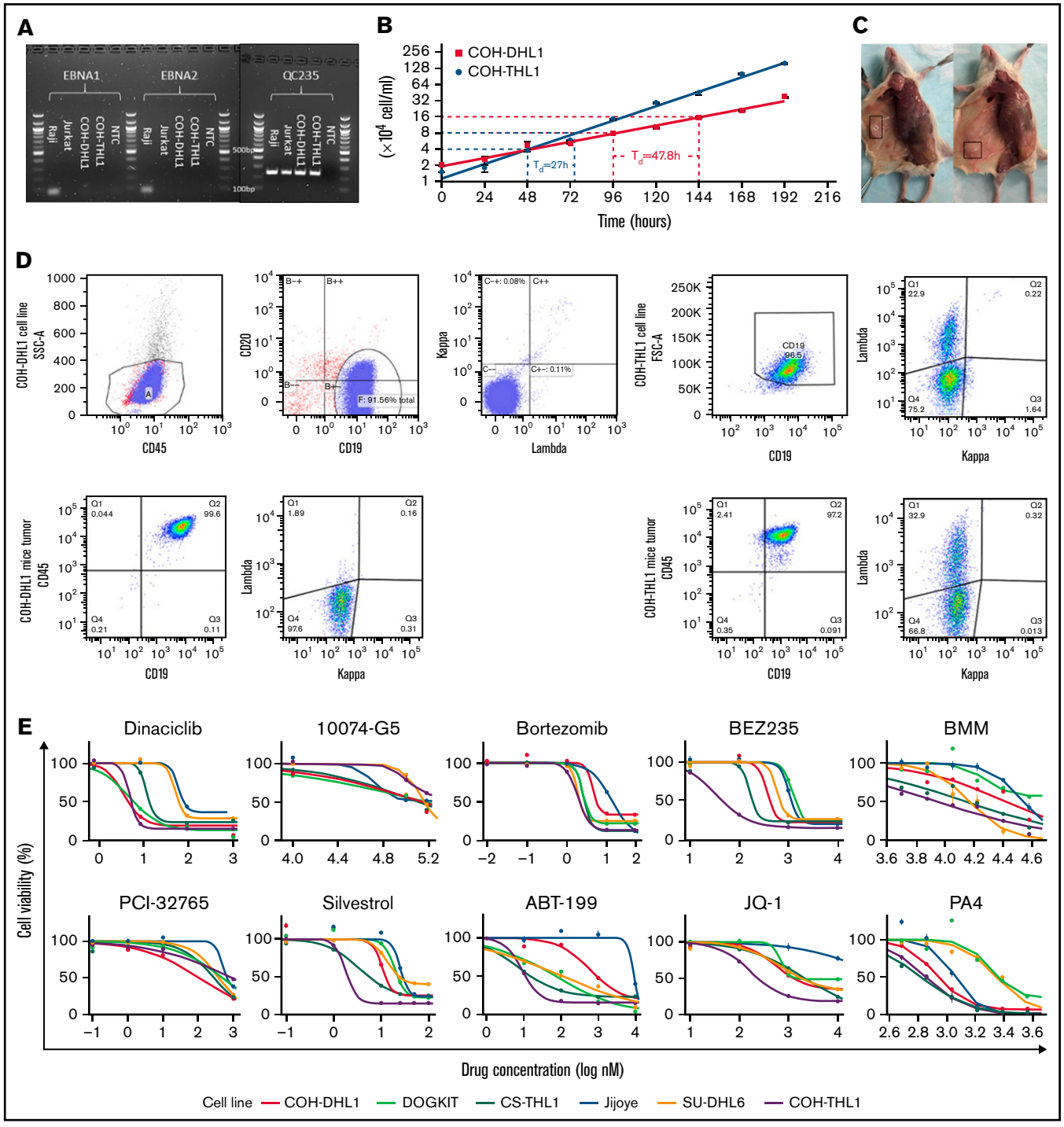

Figure 1. Characterization and validation of the D/THL cell lines. (A) EBNA (Epstein–Barr virus nuclear antigen 1) polymerase chain reaction assay of COH cell lines with negative control, an EBV-positive (Raji) and EBV-negative (Jurkat) cell line. QC235 is a positive control separately performed to confirm that DNA samples are amplifiable. (B) Growth plot for COH-DHL1 and COH-THL1 cell lines within 8 days with y-axis $\log_2$ transformed and doubling time (T_d) labeled. (C) Representative figure of mice with tumor highlighted in the box after subcutaneous injection of COH-DHL1 (left) and COH-THL1 (right). (D) Flow cytometry results of the 2 cell lines with their mice tumors. (E) Sigmoidal dose–response curves showing responses of 6 cell lines to 10 different drugs.

Berbamine[11] and its derivative PA4 through inhibition of the $Ca2^{+}$/calmodulin-dependent protein kinase γ12; and (4) inhibit the transcriptional activity of MYC by disrupting its binding site using 10074-G5.[13] Silvestrol was highly potent at the nM range. PA4 was quite promising (supplemental Table 1), whereas JQ1 and 10074-G5 were disappointing. Silvestrol is a flavagline that inhibits the activity of eIF4A subunit of the eIF4F complex. By inhibiting eIF4F, which is important for efficient translation of RNA containing

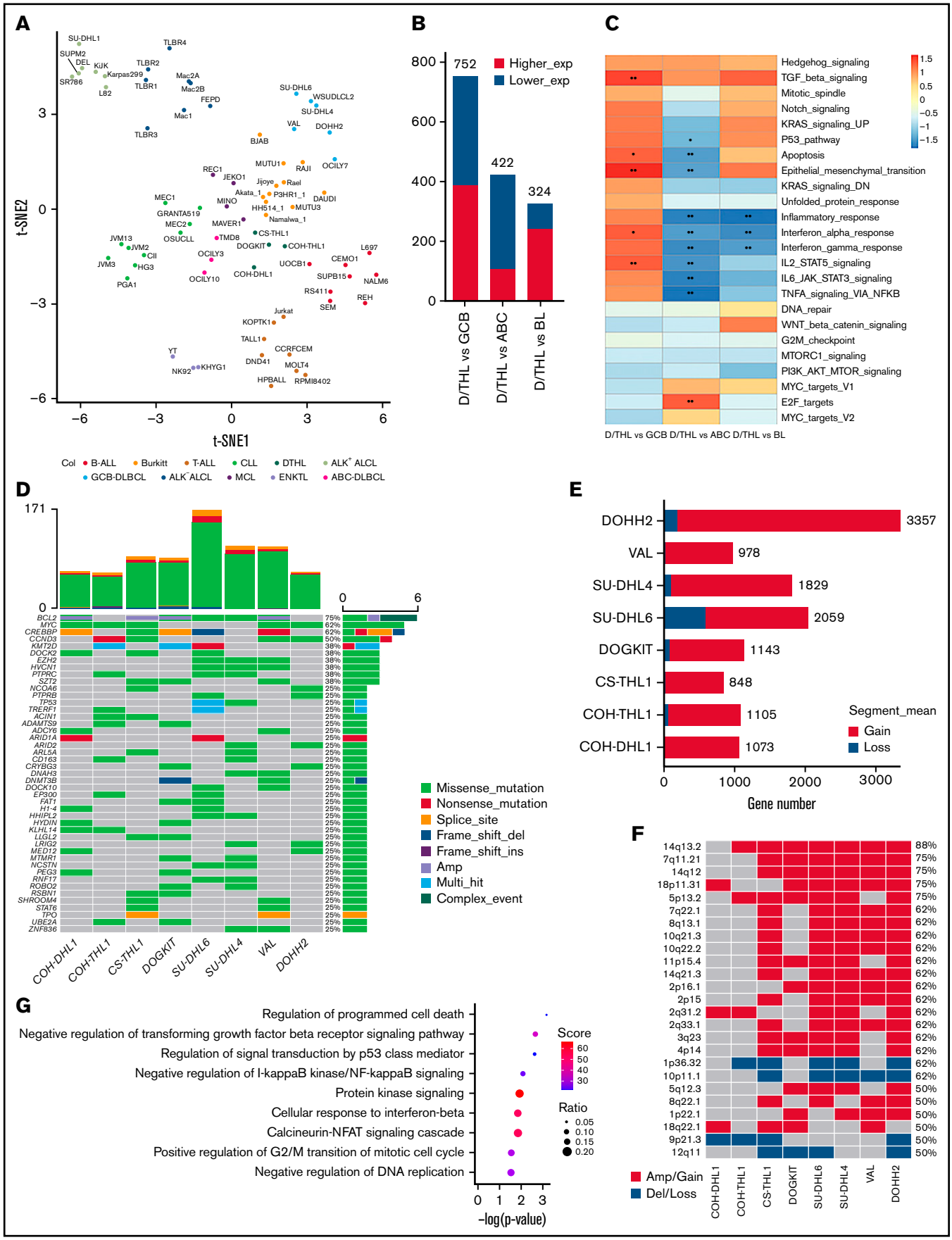
A
t-SNE1
t-SNE2
Col B-ALL Burkitt T-ALL CLL DTHL ALK+ ALCL
GCB-DLBCL ALK- ALCL MCL ENKTL ABC-DLBCL
B
752
422
324
Higher_exp
Lower_exp
D/THL vs GCB
D/THL vs ABC
D/THL vs BL
C
Hedgehog_signaling
TGF_beta_signaling
Mitotic_spindle
Notch_signaling
KRAS_signaling_UP
P53_pathway
Apoptosis
Epithelial_mesenchymal_transition
KRAS_signaling_DN
Unfolded_protein_response
Inflammatory_response
Interferon_alpha_response
Interferon_gamma_response
IL2_STAT5_signaling
IL6_JAK_STAT3_signaling
TNFA_signaling_VIA_NFKB
DNA_repair
WNT_beta_catenin_signaling
G2M_checkpoint
MTORC1_signaling
PI3K_AKT_MTOR_signaling
MYC_targets_V1
E2F_targets
MYC_targets_V2
D/THL vs GCB D/THL vs ABC D/THL vs BL
D
Missense_mutation
Nonsense_mutation
Splice_site
Frame_shift_del
Frame_shift_ins
Amp
Multi_hit
Complex_event
COH-DHL1
COH-THL1
CS-THL1
DOGKIT
SU-DHL6
SU-DHL4
VAL
DOHH2
E
DOHH2 3357
VAL 978
SU-DHL4 1829
SU-DHL6 2059
DOGKIT 1143
CS-THL1 848
COH-THL1 1105
COH-DHL1 1073
Segment_mean
Gain
Loss
Gene number
F
Amp/Gain
Del/Loss
G
Regulation of programmed cell death
Negative regulation of transforming growth factor beta receptor signaling pathway
Regulation of signal transduction by p53 class mediator
Negative regulation of I-kappaB kinase/NF-kappaB signaling
Protein kinase signaling
Cellular response to interferon-beta
Calcineurin-NFAT signaling cascade
Positive regulation of G2/M transition of mitotic cell cycle
Negative regulation of DNA replication
Score
Ratio
-log(p-value)

Figure 2.

G-quadruplex structures such as MYC and BCL2, silvestrol has shown powerful activities in human breast and prostate cancer xenograft models,[14] as well as MYC-induced lymphomagenesis.[15] All D/THL cell lines studied here were highly sensitive to silvestrol, indicating its potential as a powerful treatment option for D/THL.

Interestingly, the proteasome inhibitor bortezomib[16] was a potent drug at the nM level, whereas the BTK inhibitor PCI-32765 and PI3K inhibitor BEZ235 required substantially higher concentrations. Dinaciclib, a novel drug that inhibits cyclin-dependent kinases CDK1, 2, 5, and 9,[17] was also highly effective, especially for COH-DHL1, COH-THL1, and DOGKIT. This is consistent with the reported essential role of CDK9-mediated transcriptional elongation for tumor maintenance[18] in a genetically defined MYC-driven model of hepatocellular carcinoma and downregulation of MCL-1[19] and MYC[20] after CDK9 inhibition. In general, the COH cell lines were more responsive to most of the drugs. DHL6 tended to be more resistant, and Jijoye, which is a BL, had a different pattern of responses and tended to be the most resistant.

The 4 D/THL cell lines showed an average of 76 mutations with the most frequent alterations of *MYC, BCL2*, and *CREBBP* (supplemental Figure 3 and supplemental Table 2). Gene expression profiling of the 4 cell lines along with 74 other lymphoma/leukemia cell lines (supplemental Table 3) showed that their transcriptome profiles were close to BL, diffuse large B-cell lymphoma (DLBCL), and mantle cell lymphoma lines (Figure 2A and supplemental Figure 4A) with the highest similarity to BL cell lines (Figure 2B and supplemental Table 4). Gene set enrichment analysis showed enrichment of reported double-hit signature genes[21] in D/THL compared with DLBCL and BL (supplemental Figure 4B-C). Differentially expressed genes in D/THL vs BL were significantly ($P < .05$) negatively enriched in the inflammatory response and interferon α and γ response, which was also true vs activated B-cell like-DLBCL. However, the opposite was observed when compared with germinal center B-cell like (GCB)-DLBCL, in which enrichment in multiple pathways such as the TP53 pathway, apoptosis, epithelial–mesenchymal transition, TGFB, and IL2/STAT5 signaling was also observed for D/THL (Figure 2C). The more prominent activation of the interferon pathways in BL could be related to the presence of EBV[22] in many BL lines or more activation of endogenous retroviral elements in the BL cells.[23]

We included 4 additional reported HGBL-D/TH cell lines (SU-DHL6, SU-DHL4, VAL, and DOHH2)[2] for genetic analysis. These cell lines showed more genetic abnormalities than the COH cell lines (Figure 2D-F), indicating that many acquired changes may have occurred because of in vitro culture, and it is important to use lines with low passage numbers for study to avoid the noise from the artifactual changes. A few frequently mutant genes (eg, *CREBBP*, *EZH2*, *KMT2D*, and *EP300*) in these cell lines were shared with follicular lymphoma and GCB-DLBCL, consistent with the tumor derivation from GCB cells and the hypothesis that after acquiring the *BCL2* translocation, the cells may evolve toward a GCB-like lymphoma with the acquisition of these mutations[24] (Figure 2D). The acquisition of the MYC translocation then drives the evolution to HGBL-D/TH in concert with the BCL2 translocation. There are other detected mutations (eg, *TP53*, *PIM1*, *BAX*, and *CCND3*) that may arise before or after the *MYC* translocation and further enhance MYC-driven cell cycle progression and cell survival. Importantly, our recent study found that HGBL-D/TH cases with *TP53* abnormalities have a much worse prognosis.[25] GISTIC (Genomic Identification of Significant Targets in Cancer) identified 25 minimal common regions (MCRs) with genomic copy number abnormalities in $\geq$4 samples (Figure 2F). Among these, loss of 9p21 harboring *CDKN2A* and *CDKN2B* and gain of 2q31 were found in both COH cell lines; gains of 18p11 and 18q22 were in COH-DHL1; gains of 5p13 and 14q13 with *NFKBIA*; and loss of 1p36 containing *TNFRSF14* were in COH-THL1. Genes in these MCRs are enriched in biological processes, including apoptosis, cell cycle and cytokine, TP53, TGFβ (transforming growth factorβ) receptor, NF-κB (nuclear factor kappa B), protein kinase A, and IFN-β (interferon β) signaling (Figure 2G). We can envision that with a sufficient number of cell lines, we can further dissect different genetic/biological subgroups in vitro.

In conclusion, we established 2 new D/THL cell lines, COH-DHL1 and COH-THL1, and described their genetic characteristics as well as those of 2 additional cell lines, CS-THL1 and DOGKIT. We studied the sensitivity to 10 targeted agents using a D/THL cell line panel and demonstrated the potential of cell line models in preclinical studies of novel therapeutic agents that may benefit patients with HGBL-D/TH.

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Figure 2 (continued) Gene characterization of 4 D/THL cell lines compared with the other lymphoma/leukemia cell lines. (A) *t*-SNE clustering of the COH cell lines along with other 74 cell lines of 11 types. (B) The number of differentially expressed genes ($|\log_2$fold change$| > 1$, false discovery rate < 0.1) for contrasts between D/THL and activated B-cell like (ABC)-DLBCL, GCB-DLBCL, or BL. (C) Heatmap showing gene set enrichment analysis results with hallmark gene sets for differentially expressed genes between D/THL , ABC-DLBCL, GCB-DLBCL, and BL. Gradient colors represent the net enrichment score. *$P < .05$; **$P < .01$. (D) Waterfall plot showing the recurrent mutations in the 4 D/THL cell lines and the other 4 double-hit GCB-DLBCL cell lines. Different colors represent different types of mutations or CNA. The top bar plot shows the total number of mutations in each sample. The right bar plot shows the number of different alterations for each gene labeled by the percentage of samples that have genetic alteration in the gene. "Multi_Hit" means genes with cooccurring mutations of different types. "Complex_Event" means genes with both mutations and CNAs. (E) Bar plot showing expressed gene number covered by regions with copy number gain or loss of D/THL cell lines studied here along with another 4 double-hit cell lines. (F) Heatmap showing the distribution of the MCRs identified by GISTIC among the 8 cell lines. (G) The significant gene ontology (GO) terms ($P < .05$) enriched by the expressed genes in the MCRs ranked according to $-\log_{10}$ (*P* values) from top to bottom. The color and size of the dots indicate the enrichment score and ratio between gene number in MCRs and gene number in each GO term, respectively.

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References

1. Smith AD, Roda D, Yap TA. Strategies for modern biomarker and drug development in oncology. *J Hematol Oncol.* 2014;7(1):70.
2. Drexler HG, Eberth S, Nagel S, MacLeod RA. Malignant hematopoietic cell lines: in vitro models for double-hit B-cell lymphomas. *Leuk Lymphoma.* 2016;57(5):1015-1020.
3. Li W, Gupta SK, Han W, et al. Targeting MYC activity in double-hit lymphoma with MYC and BCL2 and/or BCL6 rearrangements with epigenetic bromodomain inhibitors. *J Hematol Oncol.* 2019;12(1):73.
4. Drexler HG, Quentmeier H. The LL-100 cell lines panel: tool for molecular leukemia-lymphoma research. *Int J Mol Sci.* 2020;21(16):5800.
5. Kiefer T, Schüler F, Knopp A, et al. A human Burkitt's lymphoma cell line carrying t(8;22) and t(14;18) translocations. *Ann Hematol.* 2007;86(11):821-830.
6. Cinar M, Rosenfelt F, Rokhsar S, et al. Concurrent inhibition of MYC and BCL2 is a potentially effective treatment strategy for double hit and triple hit B-cell lymphomas. *Leuk Res.* 2015;39(7):730-738.
7. Roberts AW, Huang D. Targeting BCL2 with BH3 mimetics: basic science and clinical application of venetoclax in chronic lymphocytic leukemia and related B cell malignancies. *Clin Pharmacol Ther.* 2017;101(1):89-98.
8. Koh CM, Sabò A, Guccione E. Targeting MYC in cancer therapy: RNA processing offers new opportunities. *BioEssays.* 2016;38(3):266-275.
9. Roderick JE, Tesell J, Shultz LD, et al. c-Myc inhibition prevents leukemia initiation in mice and impairs the growth of relapsed and induction failure pediatric T-ALL cells. *Blood.* 2014;123(7):1040-1050.
10. Wiegering A, Uthe FW, Jamieson T, et al. Targeting translation initiation bypasses signaling crosstalk mechanisms that maintain high MYC levels in colorectal cancer. *Cancer Discov.* 2015;5(7):768-781.
11. Gu Y, Chen T, Meng Z, et al. CaMKII γ, a critical regulator of CML stem/progenitor cells, is a target of the natural product berbamine. *Blood.* 2012;120(24):4829-4839.
12. Gu Y, Zhang J, Ma X, et al. Stabilization of the c-Myc protein by CAMKIIγ promotes T cell lymphoma. *Cancer Cell.* 2017;32(1):115-128.e7.
13. Yin X, Giap C, Lazo JS, Prochownik EV. Low molecular weight inhibitors of Myc-Max interaction and function. *Oncogene.* 2003;22(40):6151-6159.
14. Cencic R, Carrier M, Galicia-Vázquez G, et al. Antitumor activity and mechanism of action of the cyclopenta[b]benzofuran, silvestrol. *PLoS One.* 2009;4(4):e5223.
15. Alinari L, Prince CJ, Edwards RB, et al. Dual targeting of the cyclin/Rb/E2F and mitochondrial pathways in mantle cell lymphoma with the translation inhibitor silvestrol. *Clin Cancer Res.* 2012;18(17):4600-4611.
16. Chen D, Frezza M, Schmitt S, Kanwar J, Dou QP. Bortezomib as the first proteasome inhibitor anticancer drug: current status and future perspectives. *Curr Cancer Drug Targets.* 2011;11(3):239-253.
17. Kumar SK, LaPlant B, Chng WJ, et al; Mayo Phase 2 Consortium. Dinaciclib, a novel CDK inhibitor, demonstrates encouraging single-agent activity in patients with relapsed multiple myeloma. *Blood.* 2015;125(3):443-448.
18. Huang CH, Lujambio A, Zuber J, et al. CDK9-mediated transcription elongation is required for MYC addiction in hepatocellular carcinoma. *Genes Dev.* 2014;28(16):1800-1814.
19. Gregory GP, Hogg SJ, Kats LM, et al. CDK9 inhibition by dinaciclib potently suppresses Mcl-1 to induce durable apoptotic responses in aggressive MYC-driven B-cell lymphoma in vivo. *Leukemia.* 2015;29(6):1437-1441.
20. Moharram SA, Shah K, Khanum F, Marhäll A, Gazi M, Kazi JU. Efficacy of the CDK inhibitor dinaciclib in vitro and in vivo in T-cell acute lymphoblastic leukemia. *Cancer Lett.* 2017;405:73-78.
21. Ennishi D, Jiang A, Boyle M, et al. Double-hit gene expression signature defines a distinct subgroup of germinal center B-cell-like diffuse large B-cell lymphoma. *J Clin Oncol.* 2019;37(3):190-201.
22. Liu X, Sadaoka T, Krogmann T, Cohen JI. Epstein-Barr virus (EBV) tegument protein BGLF2 suppresses type I interferon signaling to promote EBV reaction. *J Virol.* 2020;94(11):e00258-e20.
23. Sutkowski N, Chen G, Calderon G, Huber BT. Epstein-Barr virus latent membrane protein LMP-2A is sufficient for transactivation of the human endogenous retrovirus HERV-K18 superantigen. *J Virol.* 2004;78(14):7852-7860.
24. Cucco F, Barrans S, Sha C, et al. Distinct genetic changes reveal evolutionary history and heterogeneous molecular grade of DLBCL with *MYC/BCL2* double-hit. *Leukemia.* 2020;34(5):1329-1341.
25. Song JY, Perry AM, Herrera AF, et al. Double-hit signature with TP53 abnormalities predicts poor survival in patients with germinal center type diffuse large B-cell lymphoma treated with R-CHOP. *Clin Cancer Res.* 2021;27(6):1671-1680.