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LETTER TO THE EDITOR

In vitro anti-lymphoma activity of the first-in-class pan-NOTCH transcription inhibitor CB-103

Somatic mutations leading to activation of Notch signalling are recurrent in different blood cancers, including T-cell acute lymphoblastic leukaemia (T-ALL), chronic lymphocytic leukaemia (CLL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL).[1–4] In mature B-cell lymphomas, the mutations most commonly occur in NOTCH1 and NOTCH2 genes and less frequently in genes coding for negative regulators of the pathway (such as DTX1, SPEN, FBXW7, MAML2). The presence of these mutations identifies subgroups of patients with similar clinical and biologic features. The genetically defined DLBCL subtype BN1 contains mostly activated B-cell-like (ABC) cases, and it is characterized by *NOTCH1* mutations and active Notch signalling.[2,5] *NOTCH2* mutations, alongside *BCL6* translocations, are characteristic of the BN2/C5 subtype, containing both ABC and germinal center B-cell-like (GCB) DLBCL,[2] although no active Notch signalling has been observed in DLBCL cases belonging to this subtype.[5] *NOTCH2* mutations and NOTCH2 activation are common in splenic MZL.[1,5] The hope of offering personalized therapies to these patients is sustained by the availability of drugs targeting the Notch signalling. One approach is the use of γsecretase inhibitors (GSI), small molecules that block the cleavage by an aspartyl protease of the NOTCH1–NOTCH4 receptors. The cleavage is fundamental to move the Notch intracellular domain (NICD) from the membrane to the nucleus, where it recruits transcriptional cofactors, such as RBPJ, and acts as transcriptional factor. In clinics, this class of agents has shown anti-tumour activity. However, diarrhoea, due to a skewed differentiation of intestinal stem cells towards goblet cells (goblet cell metaplasia), often represents a dose-limiting toxicity.[6] CB-103 is a first-in-class, orally active, pan-Notch inhibitor that interferes with the RBPJ–NICD transcription complex.[6] CB-103 has shown in vitro and in vivo anti-tumour activity in T-ALL. Different to what is seen with GSIs, and due to its specific mechanism of action, there is preclinical and clinical evidence that CB-103 does not induce goblet cell metaplasia.[6] CB-103 is currently in phase II (NCT03422679), based on the absence of the typical toxicities associated with GSI among the patients (41 with adenoid cystic carcinoma, 16 with colorectal cancer, four with breast cancer and two with prostate cancer) enrolled in the phase I study.[7] So far, the clinical activity is represented by the achievement of long-lasting (>6 months) stable disease in multiple patients with adenoid cystic carcinoma harbouring activating NOTCH alterations[7] and a complete response in relapse/refractory T-ALL patient in combination therapy.[8]

Here, we have studied CB-103 for its anti-tumour activity in 59 established human lymphoma cell lines, one canine and two murine lymphoma cell lines (detailed methods are presented in the Supplementary Materials). After 72 h of exposure, CB-103 determined a dose-dependent response (Figure 1; Table S1) and 14 cell lines had an IC_{50} below 10 μM: 5/20 (25%) GCB DLBCL, 3/7 (43%) ABC DLBCL, 3/9 (33%) MCL, 1/3 (33%) MZL, the canine DLBCL cell line CLBCL1 and the precursor T-ALL cell line Jurkat (Table S1). The range of active concentrations was in line with what is reported in T-ALL models.[6] CB-103 sensitive cell lines were then treated with another Notch inhibitor that had undergone early clinical development, the GSI crenigacestat (LY3039478).[9] CB-103 resulted overall superior in terms of antiproliferative activity and cell death (Figures S1 and S2). CB103 induced cell death by apoptosis in over one third of the sensitive cell lines (Figure S3). For almost all the cell lines, we can see a strong caspase activation at 72 h with increasing CB-103 concentrations (500 nM, 2 μM, 8 μM or 15 μM). For some sensitive cell lines (Jurkat, HC1 and DOHH2) at some concentrations, we saw a lower caspase activation due to higher cell death occurred before the 72 h endpoint. Data were confirmed with cell cycle experiments, showing sub-G0 cell accumulation after CB-103 treatment.

Table S2 summarizes the genetic features of the sensitive cell lines. The Jurkat cell line, the most sensitive cell line and the only one with an IC_{50} below 1 μM, has a NOTCH1 activating mutation (c.4880G > A) (Figure S4). Another two sensitive cell lines (REC1 and RI-1) have gain-of-function variants on NOTCH1 and NOTCH2, respectively, affecting the PEST domain and known to sustain Notch signalling. The other sensitive cell lines had mutations in genes involved in Notch pathway or mutated in the Notch-driven DLBCL clusters, but the contribution of the Notch-related mutations in the sensitivity cannot be clearly determined.

Expression levels of two Notch targets, *HES1* and *DTX1*, did not differ between sensitive and resistant cell lines (Figure 1). Notch transcriptional network is strictly linked with that regulated by MYC,[11] and activation of MYC was present in 57% (8/14) of sensitive cell lines (Figure S4). Of note, although two sensitive cell lines (DOHH2, OCI-Ly1)

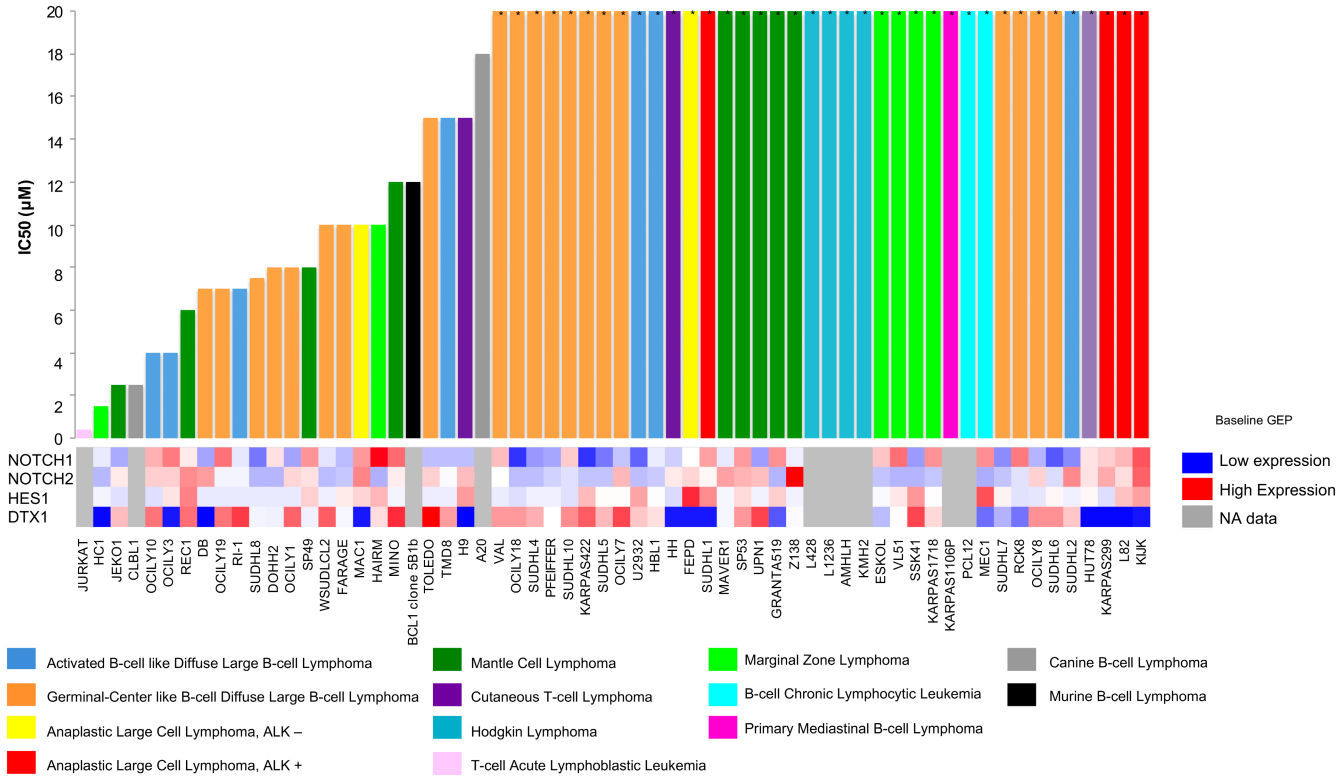

FIGURE 1 Distribution of IC_{50} values across lymphoma cell lines exposed to increasing concentrations of CB-103 or DMSO for 72 h. The heatmap shows the expression values of the NOTCH targets HES1 and DTX1. Expression values derived from previous data obtained with the HumanHT-12-v4 expression BeadChip (GSE94669).[10]

were BCL6-dependent, neither this feature nor the presence of the *BCL6* translocation were associated with response to CB-103. As reported in CLL cells,[3] genetic lesions alone might underestimate the presence of an active Notch signalling, which might be alternatively sustained by additional mechanism such as TLR signalling.[12]

We finally analysed the CB-103 induced transcriptome changes in one of the sensitive cell lines, the ABC-DLBCL OCI-Ly10, treated for 6 h with the compound (5 μM) or DMSO (Tables S2 and S3). Figure 2 shows the important biologic pathways down-regulated by CB-103, including oxidative phosphorylation, E2F and MYC targets, PI3K/AKT/MTOR. These changes were overlapping with what has been reported in two T-ALLs cell lines treated with CB-103.[6] The most up- and down-regulated protein coding genes, HLA-DRA and LMO2, respectively, are worthy of mention. The up-regulation of transcripts coding the class II major histocompatibility complex DR alpha suggests that CB-103 might counteract immune escape mechanisms.[13,14] Conversely, LMO2 is a transcription factor expressed at high levels in the vast majority of GCB DLBCL and in approximately half of ABC DLBCL, and is known to closely cooperate with Notch in normal and neoplastic cells.[15,16]

In conclusion, the pan-Notch inhibitor CB-103 have in vitro anti-tumour activity in a small subset of lymphoma cell lines derived from different lymphoma subtypes, independently from the presence of genetic lesions known to activate Notch. The activity was higher than what was achieved with a GSI. CB-103 reduced the Notch signalling and affected important biologic processes including MYC network, oxidative phosphitylation and PI3K/AKT/MTOR signalling.

AUTHOR CONTRIBUTIONS

Filippo Spriano performed experiments, performed data mining, interpreted data and co-wrote the manuscript. Chiara Tarantelli and Alberto J. Arribas performed experiments and interpreted data. Eugenio Gaudio, Andrea Rinaldi and Luca Aresu performed experiments. Luciano Cascione performed data mining. Eugenio Gaudio, Emanuele Zucca, Davide Rossi, Anastasios Stathis, Maximilien Murone and Freddy Radtke provided advice. Rajwinder Lehal co-designed and supervised the study. Francesco Bertoni co-designed the study, performed data mining, interpreted data, supervised the study and co-wrote the manuscript.

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CONFLICT OF INTEREST

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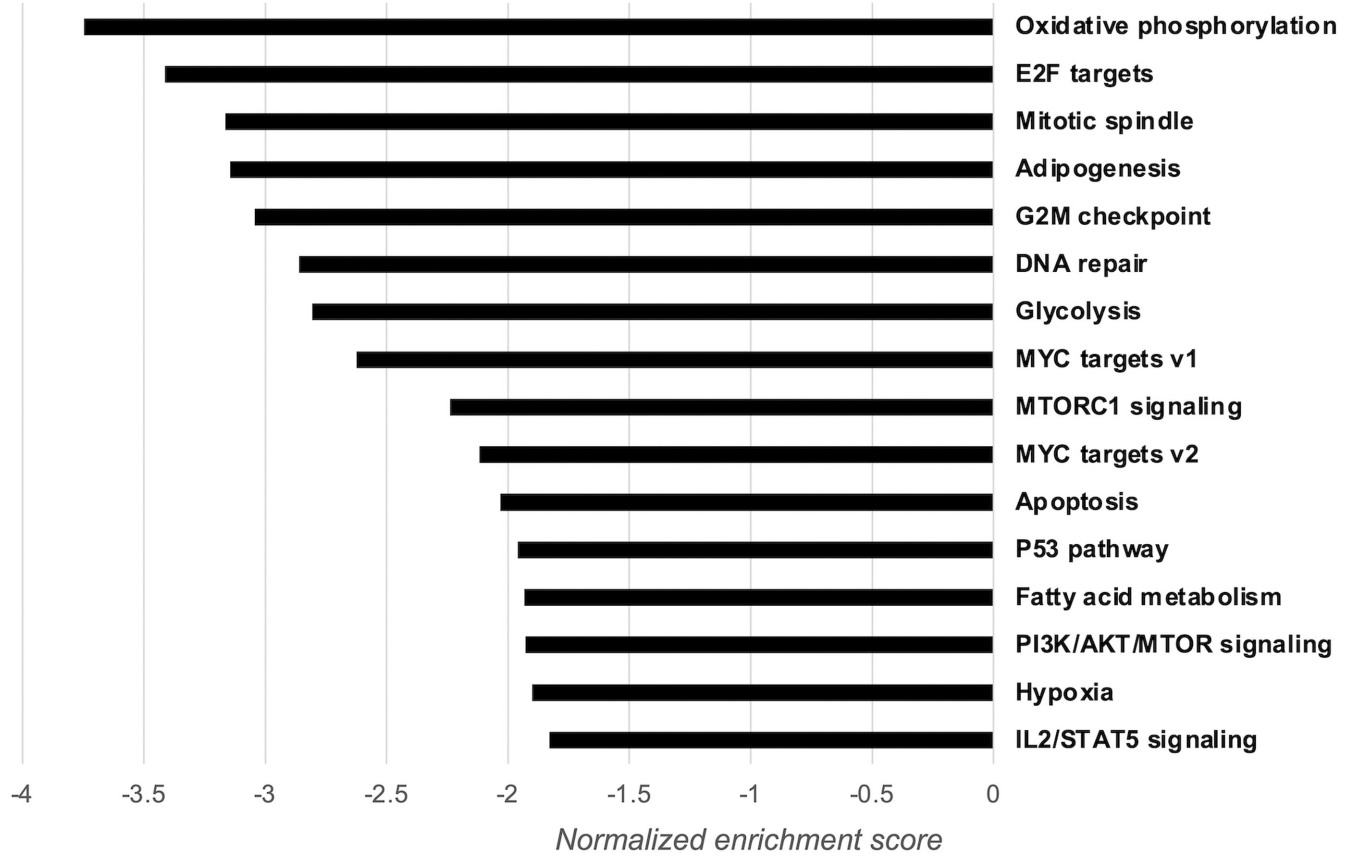

FIGURE 2 Hallmark gene sets downregulated by CB-103 as assessed by GSEA on RNA-seq data obtained in ABC DLBCL OCI-Ly10 exposed to the compound (5 μM) or DMSO for 6 h.

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DATA AVAILABILITY STATEMENT

Available in GEO (GSE152497).

PATIENT CONSENT STATEMENT

N/A.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

N/A.

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SUPPORTING INFORMATION

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