DPP8/DPP9 inhibitor-induced pyroptosis for treatment of acute myeloid leukemia

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Small-molecule inhibitors of the serine dipeptidases DPP8 and DPP9 (DPP8/9) induce a lytic form of cell death called pyroptosis in mouse and human monocytes and macrophages1–8. In mouse myeloid cells, Dpp8/9 inhibition activates the inflammasome sensor protein Nlrp1b in murine serine dipeptidases Dpp8 and Dpp9 (Dpp8/9) by Val-boroPro Casp1 mice, as Val-boroPro does not elevate serum cytokines in either a decade ago 9–11. Val-boroPro elevated the levels of a number of cytokines, including G-CSF and Cxcl1, that drive tumor-specific immunity7. We recently discovered that inhibition of two intracellular serine dipeptidases DPP8 and DPP9 (DPP8/9) induce a lytic form of cell death called pyroptosis in human AML cell lines and primary AML samples, but not in cells from many other lineages, and that these inhibitors inhibit human AML progression in mouse models. Overall, this work identifies an activator of CARD8 in human cells and indicates that its activation by small-molecule DPP8/9 inhibitors represents a new potential therapeutic strategy for AML.

Val-boroPro (Fig. 1a, also called PT-100 and talabostat) is a non-selective inhibitor of the post-proline cleaving serine pro- teases12–15 that induces antitumor immune responses in syngeneic mouse tumor models16. In mice, Val-boroPro increases the serum protein levels of several cytokines, including G-CSF and Cxcl1, and these cytokines are thought to drive tumor-specific immunity1. We recently discovered that inhibition of two intracellular serine dipeptidases Dpp8 and Dpp9 (Dpp8/9) byVal-boroPro activates the inflammasome sensor protein Nlrp1b in murine macrophages, which in turn activates pro-caspase-1 and triggers a lytic form of cell death known as pyroptosis11. This pathway is essential for the immunostimulatory activity of Val-boroPro in mice, as Val-boroPro does not elevate serum cytokines in either Casp1−/− and Nlrp1b−/− animals. On the basis of the promising anti-tumor results in mice, Val-boroPro was tested as a potential immuno-oncology agent in human clinical trials more than a decade ago10–11. Val-boroPro elevated the levels of a number of cytokines, including G-CSF, in both healthy volunteers and cancer patients3, demonstrating that Val-boroPro also activates human immune systems. We recently reported that, as in mice, Val-boroPro also induces pro-caspase-1-dependent pyroptosis in human THP-1 monocytes1. However, it remains unknown how DPP8/9 inhibition activates pro-caspase-1 in humans and whether this pathway can be harnessed for therapeutic benefit.

As DPP8/9 inhibitors are cytotoxic to THP-1 cells, which are monocyctic cancer cells cultured from the blood of a patient with AML, but not to many other cell types12, we hypothesized that DPP8/9 inhibition might be a specific vulnerability for AML cells. Val-boroPro is the most potent known inhibitor of DPP8 and DPP91, and correspondingly is also the most potent known activator of DPP8/9 inhibitor-induced pyroptosis1. We therefore first evaluated the activity of Val-boroPro across a panel of cancer cell lines (Fig. 1b,c). Consistent with our hypothesis, 12 of the 17 AML cell lines tested were sensitive to Val-boroPro as determined by CellTiter-Glo, with half-maximum inhibitory concentration (IC50) values ranging from 6 to 206 nM (Supplementary Table 1). Sensitive AML cells treated with Val-boroPro released the cytoplasmic enzyme lactate dehydrogenase (LDH) into the supernatant and stained positively for annexin V and propidium iodide (Supplementary Fig. 1), confirming that Val-boroPro was indeed inducing lytic cell death. Val-boroPro had no activity against any of the non-AML cell lines tested (Fig. 1c and Supplementary Table 2). Val-boroPro inhibits DPP4 and DPP7 in addition to DPP8/9. To confirm that these cytotoxic responses were due to DPP8/9 inhibition, we next tested two additional, albeit less potent, DPP8/9 inhibitors, l-allo-Ile-isoindoline12 and 1G24413,14, across this cell line panel (Supplementary Figs. 2 and 3). Importantly, these two compounds are structurally unrelated to Val-boroPro and are selective for DPP8/9 over DPP4 and DPP715–17. As expected, the AML cell lines that were sensitive to Val-boroPro were also sensitive to these compounds (Supplementary Fig. 3a,b and Supplementary Table 1). In contrast, these cell lines were not sensitive to the selective DPP4 inhibitor sitaglaptin or the selective DPP7 inhibitor 5385’ (Supplementary Fig. 3c,d), nor did these compounds augment the cytotoxicity of a selective DPP8/9 inhibitor (Supplementary Fig. 3e). Consistent with this inhibitor data, DPP9 knockout induced spontaneous lytic cell death in THP-1 cells1, and this effect was slightly increased in DPP8/9 double-knockout cells. Val-boroPro induced no additional cell death in DPP8/9-knockout THP-1 cells, indicating that DPP8/9 are the key targets in these human cells. We were unable to isolate DPP9-knockout MV4;11 or MOLM-13 cells, consistent with their increased sensitivities to Val-boroPro relative to THP-1 cells (Supplementary Fig. 4a). In contrast, DPP9-knockout A375 cells did not spontaneously undergo lytic cell death (Supplementary Fig. 4b,c). It should be noted that vildaglaptin, a potent inhibitor of DPP4 and a weak inhibitor of DPP8/9, was previously reported.

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to synergize with parthenolide to kill AML cells\textsuperscript{16}. However, vildagliptin did not exhibit any AML cytotoxicity on its own\textsuperscript{16}, consistent with its low affinity for DPP8/9. The mechanistic basis for its synergy with parthenolide, including whether caspase-1 and pyroptosis are involved, was not examined in this work and to date remains unknown.

Even though all of the sensitive AML cell lines responded to Val-boroPro, the extent of cell death at 48 h varied between these lines (Fig. 1b). For example, several cell lines had a >80% reduction in cell viability (MV4;11, OCI-AML2, SET-2, RS4;11 and MOLM-13), while others had only a 40–65% reduction in viability (KG1, THP-1 and NOMO-1). We speculated that these differences might reflect varying rates of pyroptosis induction, and we therefore assayed cell viability over five days (Fig. 1d–f and Supplementary Fig. 5). Consistent with this premise, MV4;11 and OCI-AML2 cells died rapidly in 1–2 days (Fig. 1d,e and Supplementary Fig. 5a), but THP-1 (Fig. 1f) and NOMO-1 cells (Supplementary Fig. 5b) required 5 days of compound treatment to achieve maximal cell killing. As expected, no cell death was observed in HEK 293T and K562 cells even after 5 days of compound treatment to achieve maximal cell killing. As expected, no cell death was observed in HEK 293T and K562 cells even after 5 days of Val-boroPro treatment (Supplementary Fig. 5c,d).

We next wanted to characterize the mechanism of DPP8/9 inhibitor-induced pyroptosis in these human cells, and in particular identify the factors that determine cell sensitivity and resistance. We first asked which genes’ expression levels are most correlated with sensitivity. This analysis, whether performed with the RNA microarray data from the Cancer Cell Line Encyclopedia (CCLE)\textsuperscript{17} across all of the cell lines (Fig. 2a), or only across the hematopoietic cell lines (Supplementary Fig. 6a), identified caspase-1 messenger RNA expression as a top predictor of Val-boroPro sensitivity. In contrast, the mRNA expression levels of DPP8 and DPP9 were not statistically different between sensitive and resistant cell lines (Supplementary Fig. 6b,c), indicating that the caspase-1 expression level, but not DPP8/9 expression levels, is a key determinant of cell sensitivity to Val-boroPro. Indeed, we found that pro-caspase-1 protein is expressed in the sensitive AML cell lines (Fig. 2b). Treatment of these cells with Val-boroPro induced cleavage of the pyroptotic substrate gasdermin D (GSDMD) and not the apoptotic substrate polyADP-ribose polymerase (PARP), demonstrating pyroptotic cell death (Fig. 2c). We confirmed that caspase-1 is required for cytotoxicity, as caspase-1-knockout OCI-AML2 (Fig. 2d,e) and THP-1 cells (Supplementary Fig. 7c,d) were resistant to Val-boroPro and l-allo-Ile-isoidoline. In addition, we found that ectopic expression of caspase-1 in CASP1\textsuperscript{−/−} THP-1 re-sensitizes the cells to Val-boroPro (Supplementary Fig. 7c,d). Pro-caspase-1 is typically activated by proximity-induced autoproteolysis into mature caspase-1 in ASC-containing inflammasomes, but pro-caspase-1 itself can be activated independent of ASC and without autoproteolysis to induce pyroptosis\textsuperscript{18–20}. DPP8/9 inhibitors activate pro-caspase-1 independent of ASC, and this form of active pro-caspase-1 cleaves GSDMD to induce pyroptosis but does not as efficiently cleave itself or pro-IL-1β\textsuperscript{1}. Indeed, no cleavage of pro-caspase-1 or pro-IL-1β was observed in any of the AML cell lines (Supplementary Fig. 7e,f). We speculate that the involvement of pro-caspase-1, rather than mature caspase-1, might explain the slow kinetics of DPP8/9 inhibitor-induced pyroptosis (days) compared to canonical
pathogen-induced pyroptosis (hours). In contrast to the sensitive cell lines, we observed little, if any, detectable pro-caspase-1 protein in the five Val-boroPro-resistant AML cell lines (Fig. 2b). Three of these cell lines, HEL, K562 and TF-1, were derived from erythroleukemias, and their low level of caspase-1 expression is consistent with their lineage, as caspase-1 expression is lowest in normal erythroid cells (Supplementary Fig. 6d)\(^1\),\(^2\). As expected, we observed no GSDMD cleavage after Val-boroPro treatment in the resistant cells, and K562 cells do not even express GSDMD (Fig. 2c). Intriguingly, HT-1080 fibrosarcoma cells expressed moderate levels of pro-caspase-1 protein but were not sensitive to Val-boroPro. We hypothesized that these cells might lack another key protein in the pathway, such as the protein activator of pro-caspase-1.

We next wanted to identify the activator of human pro-caspase-1. We predicted that the human pathway would be similar to the mouse pathway\(^3\), and therefore that DPP8/9 inhibition would activate NLRP1, the human homolog of the mouse Nlrp1b. hNLRP1 and mNlrp1b have similar domain organizations, with nucleotide-binding (NACHT), leucine-rich repeat (LRR), ‘function-to-find’ (FIIND) and CARD domains, although hNLRP1 contains an amino-terminal pyrin domain (PYD) not present in mNlrp1b (Fig. 3a)\(^\text{23}\). To determine whether hNLRP1 was required for DPP8/9 inhibitor-induced pyroptosis, we generated NLRP1\(^{−/−}\) THP-1 and MV4;11 cell lines (Supplementary Fig. 8a). Surprisingly, we found that Val-boroPro induced pyroptosis in all of these NLRP1\(^{−/−}\) cells (Supplementary Fig. 8b,c), demonstrating that NLRP1 is not required for DPP8/9 inhibitor-induced pyroptosis in humans. Intriguingly, the human genome, but not the mouse genome, encodes a protein, CARD8, that is known to bind to the CARD of caspase-1 and induce caspase-1 activation\(^\text{25,26}\), but the biological function of CARD8 has not yet been established. We next generated CARD8\(^{−/−}\) THP-1, MV4;11 and OCI-AML2 cell lines, and found that these lines were all completely resistant to Val-boroPro, as no GSDMD cleavage or cell death was observed (Fig. 3b–d). These data indicate that CARD8 and pro-caspase-1 are required for DPP8/9 inhibitor-induced pyroptosis in these cells. Consistent with a requirement for CARD8 in DPP8/9 inhibitor-induced pyroptosis, CARD8 protein is highly expressed in Val-boroPro-sensitive AML cell lines, but is not expressed in pro-caspase-1-expressing, yet Val-boroPro-resistant HT-1080 cells.

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**Fig. 2 | Caspase-1 is required for the sensitivity of AML cell lines to Val-boroPro.** a. Genes whose level of expression (from CCLE microarray data\(^1\)) distinguishes between cell lines that are sensitive and resistant to Val-boroPro. The 20 genes whose expression levels are most highly correlated with sensitivity and resistance are shown. Each row corresponds to a gene and each column corresponds to its relative expression levels in each cell line. The top ten genes are highly expressed in sensitive cell lines, and the bottom ten genes are highly expressed in resistant cell lines. b. Immunoblots of lysates from the indicated sensitive and resistant cell lines, showing levels of CASP1, CARD8, GSDMD and the loading control GAPDH. c. Immunoblots of lysates from AML cell lines treated with or without Val-boroPro for 24h, showing levels of full-length (FL) and cleaved (CL) PARP and GSDMD. See Supplementary Fig. 15 for full gel images of cropped gels. d. Immunoblots of lysates from OCI-AML2 cells treated with sgRNAs to CASP1 (control), showing levels of pro-CASP1. The immunoblot shown is representative of three independent experiments. e. Cell viability of control and caspase-1-knockout OCI-AML2 cells after treatment for 24 h with increasing concentrations of Val-boroPro (top) or l-allo-Ile-isoindoline (bottom). Data are means ± s.e.m. of three independent biological experiments. Full gel images of cropped gels are shown in Supplementary Fig. 15.
We next wondered whether CARD8 and caspase-1 expression is sufficient to confer sensitivity to DPP8/9 inhibitors. We therefore transiently transfected CARD8 or NLRP1 into HEK 293T cells stably expressing human caspase-1 and gasdermin D (to induce pyroptosis and not apoptosis), and then treated these cells with Val-boroPro. We found that expression of CARD8, but not NLRP1, rendered the cells sensitive to Val-boroPro-induced cytotoxicity (Fig. 3e,f). The FIIND of NLRP1 undergoes autoproteolytic cleavage, and this cleavage is required for activation of murine Nlrp1b and anthrax lethal toxin. Similarly, the FIIND of CARD8 also undergoes autoproteolytic cleavage and is required for Val-boroPro-induced activation, as autoproteolytic-deficient S297A mutant CARD8 did not confer sensitivity to Val-boroPro (Fig. 3e,f). Like the activation of mNlrp1b by DPP8/9 inhibitors, proteasome inhibition blocks the activation of CARD8 (Fig. 3g). In this experiment, cells were treated for only 6 h rather than 24 h due to the induction of apoptosis by bortezomib over longer intervals. Interestingly, we...
observed a marked disappearance of the N-terminal fragment of CARD8 after Val-boroPro treatment (Fig. 3b–e), particularly in OCI-AML2 cells and in HEK 293T transfections. In contrast, more of the C-terminal fragment remained. Overexpression of the C-terminal region of CARD8, but not the N-terminal region, is toxic to HEK 293T cells expressing caspase-1 (Fig. 3b). We speculate that DPP8/9 inhibitors induce proteasomal degradation of the CARD8 N terminus, freeing the C terminus to activate pyroptosis, but the details of this mechanism warrant future study. Overall, these data indicate that CARD8 and CASP1 expression are sufficient for DPP8/9 inhibitor-induced cytotoxicity.

These data suggest that DPP8/9 inhibitors, in addition to eliciting indirect anti-tumor immune responses, might also have direct anticancer activity against AML in vivo. We therefore tested the efficacy of Val-boroPro against disseminated MV4;11 leukemia cells in immunodeficient non-obese diabetic–severe combined immunodeficient (NOD–SCID) Il2rg−/− (NSG) mice. In this model, MV4;11 cells stably expressing the firefly luciferase gene were injected into the tail vein of mice. Five days after inoculation, animals were randomized into control and treatment groups, and animals in the treatment group were given a Val-boroPro regimen that was well tolerated by immunocompetent mice in previous studies. The two groups had similar disease burdens at the start of treatment (Supplementary Fig. 9a). Val-boroPro afforded a 97% reduction in tumor burden relative to the vehicle control at the end of drug treatment as measured by bioluminescence (Fig. 4a,b and Supplementary Fig. 9b). In addition, Val-boroPro-treated mice experienced a significant increase in survival over control-treated animals (Figs. 4c, 24.5-day median extension in survival, P = 0.005, log-rank test). This efficacious dosing was well tolerated, as we observed no significant effects on peripheral blood cell counts, serum chemistry or body weight in these mice (Supplementary Fig. 9c–f). In similarly dosed wild-type C57BL/6J mice, we similarly observed no significant effects on peripheral blood counts or body weight (Supplementary Fig. 10). To confirm that the in vivo efficacy of Val-boroPro was due to pyroptosis of the cancer cells themselves, we performed a similar experiment using CASP1−/− MV4;11 leukemia cells. Val-boroPro had absolutely no efficacy against these cells in vivo (Fig. 4d,e), thereby establishing caspase-1-mediated pyroptosis of the AML cells as the basis for the in vivo efficacy. We next tested the efficacy of the more selective, albeit less potent, DPP8/9 inhibitor compound 8j28 in this leukemia model. We found that the compound 8j significantly inhibited leukemia progression (Supplementary Fig. 11), further confirming that DPP8/9 are the key targets mediating this response.

Primary AMLs express the highest median level of caspase-1 mRNA of all cancer types assessed in The Cancer Genome Atlas (Supplementary Fig. 12a)22–26. Moreover, high caspase-1 expression in AML is correlated with lower overall survival (Supplementary Fig. 12b)22,14, indicating that caspase-1 is likely present in cancers with poor prognoses. Similar to the AML cell lines, we found that primary AML cells were sensitive to Val-boroPro (Supplementary Fig. 13a,b). We confirmed that caspase-1 and CARD8 proteins are present in primary AML samples (Supplementary Fig. 14a,b), and that Val-boroPro induced cleavage of GSDMD into its pyroptotic p30 fragment without any evidence of apoptotic PARP cleavage (Supplementary Fig. 14b). As expected, no pro-caspase-1 cleavage was observed in any of the primary AML samples. We next determined the impact of Val-boroPro in a patient-derived xenograft (PDx) model of AML (categorized as FAB M1 AML, see Supplementary Table 3 for molecular characterization). After 10 days of dosing, Val-boroPro significantly reduced the number of human AML cells in peripheral blood by ~75% relative to the vehicle control (Fig. 4f). Moreover, Val-boroPro slowed weight loss associated with leukemia progression in this PDx model (Fig. 4g).

Collectively, these data indicate that selective inhibition of DPP8/9 is sufficient for cytotoxic activity against AML, and argues for the development of a potent and selective DPP8/9 inhibitor as a therapeutic agent for AML. An important caveat, however, is that the mechanism of action of DPP8/9 inhibitors in humans (CARD8) is different than their mechanism of action in mice (Nlrp1b), and therefore mouse models may not accurately predict the therapeutic window of DPP8/9 inhibitors in humans.
For example, DPP8/9 inhibitors may induce pyroptosis in different primary cell lineages or at different doses in humans and in mice. Although we found that non-AML human cancer cell lines (Fig.1 and Supplementary Table 2) and primary T-ALLs (Supplementary Fig. 14b,c) were completely resistant to Val-boroPro, primary human B-ALL (Supplementary Fig. 14d,e) and primary human cord blood CD34+ cells (Supplementary Fig. 14f) were sensitive to Val-boroPro. This may indicate that B-ALLs are also amenable to pyroptosis-mediated killing, but it could also suggest increased toxicity in humans compared with mice. Regardless, Val-boroPro reached serum concentrations in humans above the IC_{90} of human AML cells (>100 nM) without reaching a maximum tolerated dose, indicating that Val-boroPro itself is potentially a viable anti-AML agent. A more selective DPP8/9 inhibitor, or dosing DPP8/9 inhibitors after depleting hematopoietic cells (as would be achieved with the standard of care for AML), might further mitigate potential toxicities as well.

In summary, here we have discovered that CARD8 mediates DPP8/9 inhibitor-induced pyroptosis in human myeloid cells. CARD8 has long been implicated in innate immunity; here, we report that it acts as an ‘inflammasome’ sensor to activate caspase 1 in response to a specific stimulus. The anticancer potential of this pathway, which includes both direct cytotoxicity to AML cells and indirect induction of immune responses to solid tumors, warrants future investigations.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41591-018-0082-y.

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Author contributions

D.A.B. conceived and directed the project, performed experiments, analyzed data and wrote the paper; D.C.J., C.Y.T., M.C.O., A.J.C. and S.D.R. performed experiments and analyzed data; D.C.J., M.C.O., F.C.B., C.R. and E.P. performed xenograft experiments; E.D.S. advised on the xenograft experiments. A.K. provided cell biology, mouse xenograft and leukemia expertise.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Cloning. Single guide RNA (sgRNAs) were designed using the Broad Institute’s web portal17 (http://www.broadinstitute.org/rnai/public/analysis-tools/sgrna-design) and cloned into the lentiGuide-Puro vector (Addgene no. 52963) as described previously18. The sgRNA sequences are listed in Supplementary Table 4. cDNA coding for full-length human CASP1 (Origene) was cloned with a C-terminal HA tag into pLmducer20 vector (Addgene) and with a stop codon into a modified version of the pLEX_S97 vector (Addgene) with a hygromycin resistance marker using Gateway technology (Thermo Fisher Scientific). cDNA coding for full-length human GSDMD (Dharmacon) was cloned into the pLEX_S97 vector (Addgene) with a C-terminal V5 tag. cDNAs coding for full-length NLRP1 and CARD8 were purchased from Origene and RFP from Addgene and cloned with modified version of the pLEX_307 vector (Addgene) with a hygromycin resistance (Origene) was cloned with a

described previously34. The sgRNA sequences are listed in Supplementary

Reagents and antibodies. Val-boroPro19, 1,2-allyl-(d)-isoadoline17, 8j, and 35s25 were synthesized according to previously published protocols. For cell culture experiments, Val-boroPro was resuspended in DMSO containing 0.1% DMSO to prevent compound cyclization. For in vivo experiments, Val-boroPro was reconstituted and stored in sterile 0.01 M HCl at a concentration of 2 mg ml$^{-1}$. Immediately in vivo administration, the 2 mg ml$^{-1}$ stocks of sterile Val-boroPro were diluted to 20 µg per ml in sterile PBS (pH=7.4). LPS was purchased from Santa Cruz Biotechnology, nigerin and docycycline from the Cayman Chemical Company, doxycycline from (Dharmacon) and DsRed2 (Clontech) were obtained from DermaScience Laboratories, transposase from Enzo Life Sciences, and sitaglitin from Sigma. Antibodies used were: HCD45 (no. 304036, BioLegend, V510 conjugate, clone HI30), hCD45 (no. 304012, BioLegend, APC conjugate, clone HI30), mCD45 (no. 103108, BioLegend, FITC conjugate, clone 30-F11), hCD3 (no. 304040, BioLegend, FITC conjugate, clone UC7H1), hCD3 (B8231, BioCell, clone UC7H1), hCD33 (no. 366618, BioLegend, PE-Cy7 conjugate, clone 567.6), human caspase-1 (no. 2225, Cell Signaling Technology), DPP9 (ab20809, Abcam), GAPDH (clone 14C10, Cell Signaling Technology), GSDMD (NB2-33422, Novus Biologicals), IL-1β (Clon 2805, R&D Systems), PARP (no. 9542, Cell Signaling Technology), NLRP1 (AB_10891878, R&D Systems), CARD8 N-term (no. ab198558, Abcam) and CARD8 C-term (no. ab24186, Abcam).

Cell culture. A375, HEK 293T, THP-1, RS4.11, HT-1080, Jurkat, U937, and MCF-7 cells were purchased from ATCC. SET-2, HL-60, KGI, MOLM-13, MV4;11, NOMO1, NB4, OCI-AML2, OCI-AML3, HEL, KASUMI-1, K562, U937 SET2 and Jurkat cells were gifts from J. Schale (Charité – Universitätsmedizin Berlin, Germany) and were verified using STR genotyping (Genetica DNA Laboratories). HEK 293T, DA0Y, HT-1080, IMR, JURKAT, MCF-7, ONS-76, RDES and TC-1 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS). THP-1, RS4.11, HL-60, KGI, MOLM-13, MV4;11, NOMO1, NB4, OCI-AML2, HEL, KASUMI-1, K562, U937 SET2 and Jurkat cells were grown in RPMI-1640 medium with 10% FBS. CD45+ human cord blood (CB) cells (STEMCELL Technologies, catalog no. 70006.3) were thawed in IMDM with 15% FBS and DNase1 (1 mg ml$^{-1}$) and grown in IMDM supplemented with 10% FBS, 1× penicillin/streptomycin, 100 µg ml$^{-1}$ TPO, 100 ng ml$^{-1}$ SCF and 10 µg ml$^{-1}$ IL-3 (PeproTech 200-03) and 50 ng ml$^{-1}$ G-CSF (PeproTech 300-3C). 100 µg ml$^{-1}$ val-boroPro was diluted to 20 µg ml$^{-1}$ in IMDM and added to the CM to generate bioluminescent MV4;11 cells for in vivo studies. To generate bioluminescent MV4;11 cells for in vivo studies, the PMMP-luc-MV4;11 cells (BioLegend) were transduced with the pMSCV-Puro vector (Addgene cat. no. 9816) containing the luciferase gene and the puromycin-resistant gene. MV4;11 cells were grown in IMDM supplemented with 10% FBS, 1× penicillin/streptomycin, 100 ng ml$^{-1}$ IL-3 and 50 ng ml$^{-1}$ G-CSF. Transduced MV4;11 cells were treated with val-boroPro (2.5 µg ml$^{-1}$) and stained with anti-mouse CD45 (FITC), anti-human CD45 (APC), and PI (Fisher Scientific) or DAPI (Thermo Fisher Scientific). Stained cells were sorted using a BD FACSAria II instrument (BD Bioscience) and data were analyzed using FACSDivA (BD Bioscience) and FlowJo software. The gating strategy for flow cytometry is shown in Supplementary Fig. 16.

Immunoblotting experiments. Cell lines (1.0 × 10$^6$ cells) were seeded in 6-well plates and treated with DMSO or compounds as described. THP-1 cells were also treated with LPS (10 µg ml$^{-1}$) for 24 h. After 24 h, LPS-primed cells were treated with nigerin (20 µg ml$^{-1}$) for 30 min. For primary cell western blots, primary cells were thawed and seeded in 6-well plates at 3 × 10$^5$–4 × 10$^6$ cells per well. Seeded primary cells were treated with DMSO or Val-boroPro for 24 h. Cells were washed twice in PBS (pH 7.4), resuspended in PBS and lysed by sonication. Protein concentrations were determined using the DCA Protein Assay kit (Bio-Rad). The samples were separated by SDS–PAGE, immunoblotted and visualized using the Odyssey Imaging System (Li-Cor).

Generation of stable cell lines. For generation of knockout cell lines, constructs were packaged into lentivirus in HEK 293T cells using the Fugene HD transfection reagent (Promega) and 2 µg of the vector, 2 µg pSiPA2X and 0.2 µg pmD2G. Target cells were spinfected with virus particles for 24 h in 30 °C supplemented with 8 µg ml$^{-1}$ Polybrene. After 2 days, cell viability assays were performed on CB cells immediately after thawing. Cell lines were tested for the stable expression of Streptococcus pyogenes Cas9 (Addgene no. 52962) using basicidin (5 µg ml$^{-1}$ for MV4;11, MOLM-13 and OCI-AML2; 1 µg ml$^{-1}$ for THP-1) and for stable expression of sgRNAs using puromycin (0.5 µg ml$^{-1}$ for OCI-AML2 and THP-1; 1 µg ml$^{-1}$ for MV4;11 and MOLM-13). After 10 days, the cells were collected for immunoblotting or experiments. For isolation of complete knockouts, single cells were isolated by serial dilution and expanded. To generate bioluminescent MV4;11 cells for in vivo studies, the PMMP- luc retroviral plasmid (6 µg) was transfected into HEK293T cells with the packaging vectors pMD.MLV (1.5 µg) and pCMV-VSV-G (1.5 µg) using the Fisher Luc retroviral transfection reagents. MV4;11 cells were spinfected at a multiplicity of infection of 1 for 1 h at 2,000 µg ml$^{-1}$ for 37 °C supplemented with 8 µg ml$^{-1}$ Polybrene. After 2 days, firefly luciferase-expressing cells were selected with neomycin (2 µg ml$^{-1}$) for 14 days. For the ectopic expression of CasP1 and GSDMD, the expression plasmid (2 µg) was transfected into HEK293T cells with the packaging vectors pSiPA2X (2 µg) and pCMV-VSV-G (0.2 µg) using FugeneHD transfection reagent. After 2 days, THP-1 CasP1-knockout or HEK 293T cells were spinfected with virus particles for 1 h at 1,000 µg ml$^{-1}$ with 8 µg ml$^{-1}$ Polybrene. After 2 days, THP-1 CasP1-expressing cells were selected with G418 (200 µg ml$^{-1}$), HEK 293T CasP1-expressing cells were selected with hygromycin (200 µg ml$^{-1}$) and HEK 293T GSDMD-expressing cells were selected with puromycin (1 µg ml$^{-1}$).

Gene expression analysis. Gene expression data were obtained from the CCLE (https://portals.broadinstitute.org/ccle/home1), and genes distinguishing sensitive and resistant cell lines were identified with a two-class comparison using Morphos (https://software.broadinstitute.org/morphos/).
Annexin V and PI staining. A total of $1 \times 10^6$ cells were treated with DMSO, Val-boroPro (2 μM) or etoposide (50 μM) for 24 h. The cells were then analyzed using the annexin V–FITC Apoptosis Kit (Clontech) according to the manufacturer’s protocol. The gating strategy for flow cytometry is shown in Supplementary Fig. 16.

In vivo studies. All mouse experiments with MV4;11 cells were performed at the MSKCC animal facility and approved by the institutional animal care and use committee. One million MV4;11-Luc Neo cells were injected into the tail vein of sub-lethally (200 cGy) irradiated 7-week-old female NSG mice (The Jackson Laboratory). For the Val-boroPro experiment with wild-type MV4;11 cells, 5 days after injection, the mice were randomly grouped ($n=5$ mice per group) and treated intraperitoneally with 100 μl of vehicle (1 mM HCl in PBS, pH = 7.4) or 100 μl of Vbp (20 μg per 100 μl) once daily, 5 days a week for 4 weeks. Animals were weighed daily. Tumor burden was measured on the indicated days by bioluminescence imaging using an IVIS Spectrum system (Caliper Life Sciences). After 23 days of drug dosing, peripheral blood from three mice per group (high, median and low total flux bioluminescence imaging readings) was obtained and analyzed for complete blood counts and blood chemistry by MSKCC Antitumor Assessment Core and Laboratory of Comparative Pathology, respectively. The femur, spleen, lung and liver from these mice were also collected, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin (sectioning and staining was performed by the MSKCC Antitumor Assessment Core and the Molecular Cytology Core). Survival analysis was measured from the time of cancer inoculation to the moribund state (six mice per group), and Prism 7 software (Graph Pad) was used for statistical analysis. We selected nine mice per group so that we could perform blood and tumor burden analysis on three mice and have six mice for survival analysis, similar in size to previous studies using this model. For the Val-boroPro experiment with CASPI− MV4;11 cells, the experiment was performed as described for wild-type MV4;11 cells, except dosing was stopped after three weeks due to a lack of efficacy and survival analysis was performed using all nine animals. For the experiment with the compound 8j, five days after tumor injection, the mice were randomly grouped ($n=6$ mice per group) and treated intraperitoneally with 100 μl of vehicle (1% DMSO in PBS) or 100 μl of the compound 8j (600 μg per 100 μl) once daily, 5 days a week for 4 weeks. Tumor burden was determined as described above. To evaluate Val-boroPro in C57BL/6J mice, 7-week-old female C57BL/6J mice (The Jackson Laboratory) were treated with either vehicle or Val-boroPro (2 mg kg−1) for 28 days (5 days per week for 4 weeks). Animals were weighed daily. Tumor burden in the peripheral blood was determined by FACS analysis. This study was not designed to monitor overall survival. No animals were excluded.

EnPlex assay. Selectivity profiles were determined by EnPlex as described previously.

Statistical analysis. Two-sided Student’s $t$-tests were used for significance testing unless stated otherwise. $P$ values less than 0.05 were considered to be significant. Graphs and error bars represent means ± s.e.m. of independent biological experiments unless stated otherwise. For all experiments, the investigators were not blinded. All statistical analysis was performed using GraphPad Prism 7.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All relevant data are available upon reasonable request to the corresponding author.

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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
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- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
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- Clearly defined error bars
- State explicitly what error bars represent (e.g. SD, SE, CI)

Software and code

Policy information about availability of computer code

Data collection
Flow cytometry data was obtained using FACSDiva 8.0.1 (BD Biosciences, San Jose, CA).

Data analysis
Statistical analysis were performed using PRISM 7.0 (GraphPad, La Jolla, California). Bioluminiscence imaging data were quantified with Living Image 4.5 software (Caliper Life Science, USA). Flow cytometry data was analyzed using FloJo v10. Online Morpheus and BloodSpot programs were used as described.

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data is available upon request.

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# Life sciences

## Study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample size was determined based on previous studies. This is noted in the Materials and Methods. |
|-------------|-------------------------------------------------------------------------------------------------|
| Data exclusions | No data were excluded. |
| Replication | All attempts at replication of experimental findings were reliably reproduced. |
| Randomization | Samples were allocated into groups randomly. |
| Blinding | Investigators were not blinded during data collection. |

## Materials & experimental systems

### Antibodies

Antibodies used: hCD45 (#304036, BioLegend, V510 conjugate, clone HI30), hCD45 (#304012, BioLegend, APC conjugate, clone HI30), mCD45 (#103108, BioLegend, FITC conjugate, clone 30-F11), hCD3 (#300440, BioLegend, FITC conjugate, clone UCHT1), hCD3 (BE0231, BioXCell, clone UCHT1), hCD33 (#366618, Biolegend, PECy7 conjugate, clone P67.6), human caspase-1 (#2225, Cell Signaling Technology), DPP9 (ab42080, Abcam), GAPDH (clone 14C10, Cell Signaling Technology), GSDMD (NBP2-33422, Novus Biologicals), IL-1β (Clone 2805, R&D Systems), NLRP1 (AB_10891878, R&D Systems), CARD8 N-term (#ab194585, Abcam), CARD8 C-term (#ab24186, Abcam), and PARP (#9542, Cell Signaling Technology).

### Validation

Human caspase-1, DPP9, GAPDH, GSDMD, IL-1β, and PARP are extensively used and validated in the literature, including in our previous manuscript (Okondo et al, Nature Chemical Biology, 2017). CARD8 N- and C-term and NLRP1 antibodies were validated in this manuscript using knockout cell lines and ectopic expression of the proteins. The BioLegend antibodies were validated by flow cytometric analysis, as described on the manufacturer’s website. The hCD3 Ab has been validated in numerous publications, as described on the manufacturer’s website.

### Eukaryotic cell lines

A375, HEK 293T, THP-1, RS4;11, HT-1080, Jurkat, U937, and MCF-7 cells were purchased from ATCC. SET-2, HL-60, KG1, MOLM-13, MV4;11, NOMO1, NB4, OCIAML2, OCI-AML3, HE1, KASUMI-1, TF-1, and K562 cells were purchased from...
DSMZ, DAOY, IMR5, ONS-76, RD-ES, and TC71 cells were gifts from Johannes Schulte.

Authentication

ATCC uses morphology, karyotyping, and PCR based approaches to confirm the identity of human cell lines and to rule out both intra- and interspecies contamination. These include an assay to detect species specific variants of the cytochrome C oxidase I gene (COI analysis) to rule out interspecies contamination and short tandem repeat (STR) profiling to distinguish between individual human cell lines and rule out intra-species contamination. DSMZ uses STR profiling to confirm cell line identity. Cells not from ATCC or DSMZ were verified by STR genotyping (Genetica DNA Laboratories).

Mycoplasma contamination

Cell lines were confirmed mycoplasma free using the MycoAlertTM Mycoplasma Detection Kit (Lonza).

Commonly misidentified lines

None used in this study.

Research animals

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Animals/animal-derived materials 7-week-old female non-obese diabetic–severe combined immunodeficient (NOD–SCID) Il2rg−/− (NSG) mice, 5-week-old female NOD.Cg-Prkdcscid Il2rgtm1Sug/JicTac (NOG; Taconic #NOG-F), and 7-week-old C57BL/6J female mice.

Human research participants

Policy information about studies involving human research participants

Population characteristics Data is arranged as follows: Sample ID, Malignancy, Sex, Age, Cytogenetics, Mutations: AML-1, AML, F, 44, 46,XX, DNMT3A R882H, NPM1m, FLT3 ITD; AML-2, AML, M, 62, 46,XY, DNMT3A R882H, NPM1m, NRAS G12D; AML-3, AML, F, 61, 46,XX, NPM1m, FLT3 ITD; AML-4, AML, F, 9.7, 46,XX, NRAS G12D, FLT3 ITD, MLL duplication; AML-5, AML, M, 39, Complex, AML1-ETO; AML-6, AML, M, 15, Complex, MLL-AF4, PTPN11 E76K; AML-7, AML, F, 13, 46,XX, del(6)(p21p23),t(11;19)(q23;p13.1)[16]; AML-8, AML, M, 60, 46,XY, inv(16), IDH2 R140Q; AML-9, AML, M, 56, 46,XY,del(7)(q22)[20], IDH1 R132G, DNMT3A R771*, NPM1m; AML-10, AML, M, 39, Complex, IDH R132C, FLT3 ITD, NRAS G12D, TET1 A34V; AML-11, AML, M, 31, Complex, AML1-ETO; T-ALL-1, T-ALL, F, 22, 46,XX[20], NF1 K2664fs*1, NOTCH1 deletion exons 3-27, BCOR splice site 4717+1G>A, CTCF P41fs*12, PHF6 R129*,TP53 P152L; T-ALL-2, T-ALL, M, 61, N/A, N/A, B-ALL-1, B-ALL, M, 71, N/A, N/A; B-ALLN/A, N/A, 7 [18]/46,XY [20], NRAS Q61R, ASXL1 G646fs*12, NUP98 N131I/I+18

Method-specific reporting

n/a Involved in the study

- □ ChIP-seq
- □ Flow cytometry
- □ Magnetic resonance imaging

Flow Cytometry

Plots

Confirm that:

- □ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- □ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- □ All plots are contour plots with outliers or pseudocolor plots.
- □ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation Primary samples were collected by bone marrow aspirate from consenting patients on protocol 09-141. After bone marrow aspiration, the bone marrow aspirate was passed through a 100 μM cell strainer and washed with 1X PBS. The cell suspension was layered over Ficoll-Paque PLUS and centrifuged at 1000 x g for 20 minutes. Bone marrow mononuclear cells (BM MNCs) at the interphase were transferred to a new conical tube and washed with 1X PBS. BM MNCs were resuspended in 1X PBS, 1× penicillin/streptomycin (Corning), 0.1 mM 2-mercaptoethanol, 20 ng/mL G-CSF (Peprotech 300-23), 100 ng/mL SCF
(PeproTech 300-07), 20 ng/mL IL-3 (PeproTech 200-03), 50 ng/mL FLT3-Ligand (PeproTech 300-19). For the MV4;11 cells, no tissue processing was required.

| Instrument       | We used two instruments: 1) BD FACSCanto II and 2) BD LSR Fortessa. |
|------------------|---------------------------------------------------------------------|
| Software         | Flow cytometry data was obtained using FACSDiva 8.0.1 (BD Biosciences, San Jose, CA). Flow cytometry data was analyzed using FloJo v10. |
| Cell population abundance | Not applicable.                                                       |
| Gating strategy  | The gating strategy of all relevant experiments is shown in Supplementary Figure 16. For primary human samples, cells were gated by FSC-A and SSC-A, and then gated for human CD45+ cells. Live cells were identified by gating DAPI or PI negative cells. For PI-Annexin V staining of cell lines, cells were gated by FSC-A and SSC-A, and then gated into quadrants based on PI and Annexin V staining. |

☑ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.