

Combined inhibition of XIAP and BCL2 drives maximal therapeutic efficacy in genetically diverse aggressive acute myeloid leukemia

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Aggressive therapy-resistant and refractory acute myeloid leukemia (AML) has an extremely poor outcome. By analyzing a large number of genetically complex and diverse, primary high-risk poor-outcome human AML samples, we identified specific pathways of therapeutic vulnerability. Through drug screens followed by extensive *in vivo* validation and genomic analyses, we found inhibition of cytosolic and mitochondrial anti-apoptotic proteins XIAP, BCL2 and MCL1, and a key regulator of mitosis, AURKB, as a vulnerability hub based on patient-specific genetic aberrations and transcriptional signatures. Combinatorial therapeutic inhibition of XIAP with an additional patient-specific vulnerability eliminated established AML *in vivo* in patient-derived xenografts (PDXs) bearing diverse genetic aberrations, with no signs of recurrence during off-treatment follow-up. By integrating genomic profiling and drug-sensitivity testing, this work provides a platform for a precision-medicine approach for treating aggressive AML with high unmet need.

For decades, the backbone of AML treatment has remained anthracyclines (DNA intercalators and topoisomerase II inhibitors) combined with the nucleoside analog cytarabine¹. More recently, studies have led to the approval of specific targeted inhibitors of pathogenic mutant proteins (for example, inhibitors of mutant FMS-related receptor tyrosine kinase (FLT)3, isocitrate dehydrogenase (IDH)1 and IDH2)^{2–5} and of survival pathways (for example, inhibitors of BCL2 and the hedgehog pathway)^{6,7}. Although these new therapeutics have broadened treatment options, the outlook for adverse-risk and relapsed or refractory disease remains very poor⁸. Clonal heterogeneity within any one patient and between patients, coupled with a lack of deep understanding of resistance mechanisms in pre-leukemic and leukemic stem cells are substantial barriers to improving clinical outcome^{9–14}. For adverse prognostic risk of refractory and relapsed patients, allogeneic stem cell transplantation (allo-SCT) remains the most effective curative treatment. However, only a minority of patients with AML are able to tolerate the appreciable toxicity of allo-SCT, as most are older and/or carry comorbidities. Moreover, 40–60% of patients with poor risk relapse after allo-SCT and ~10% of fit patients are refractory to any therapy. Given this unmet need, here we focused on determining the therapeutic vulnerabilities in primary human AML with the most aggressive course and poorest clinical outcome in a cohort with diverse genetic backgrounds. To identify therapeutic targets, we performed RNA-seq on functionally defined AML-engrafting cells followed by *in vitro* testing of directed libraries of small molecules based on pathways that were differentially expressed between AML-engrafting cells and normal hematopoietic stem or progenitor cells (HSPCs). A matrix of five small-molecule inhibitors were then taken forward for extensive *in vivo* testing of primary human AML samples in PDX models.

Combination treatment strategies based on AZD5582, a second mitochondrial activator of caspases (SMAC) or mitochondrial DIABLO homolog mimetic, which bivalently blocks inhibitor of apoptosis proteins (IAPs)¹⁵, and inhibition of additional patient-specific vulnerability hubs (BCL2, MCL1 or AURKB) were highly effective in vivo against a range of poor-risk leukemia samples. By linking drug sensitivity and genetic and cytogenetic information, we identified correlations between XIAP or BCL2 dependence with mutations (FLT3, IDH1, IDH2, CBL, NRAS, TET2), chromosomal aberrations (rearrangements involving MLL (KMT2A) or EVI1 (MECOM)) and tumor protein 53 (TP53) transcriptional regulatory function in the majority of cases. These findings will facilitate curative precision medicine for the most clinically aggressive AML in the future.

Results Identification of therapeutic targets in clinically aggressive adverse-risk AML. We obtained bone marrow (BM) or peripheral blood (PB) samples from 136 patients with clinically aggressive leukemias, including FLT3 wild type (WT) AML (n = 89), FLT3-mutated AML (n = 38; internal tandem duplication (ITD)+ (n = 26), tyrosine kinase domain (TKD)+ (n = 12)), myelodysplastic syndrome (n = 3), Ph+ mixed phenotype acute leukemia (MPAL) and Ph+ acute lymphoblastic leukemia (ALL) (n = 6) (patient characteristics are summarized in Supplementary Table 1). Risk stratification by European LeukemiaNet (ELN) 2010 criteria showed an excess proportion of patients with adverse-risk AML in our study population (n = 126) compared with previously reported populations of patients with AML enrolled in multicenter clinical trials (Extended Data Fig. 1a)^{16–18}. Among patients with AML, 66.7% (84 of 126) had relapsed disease or were refractory to treatment. Consistent with risk stratification, 116 of 126 patients (92.1%) failed to achieve stable remission, and 107 of 126 (84.9%) patients died of the disease (Fig. 1a). We aimed to discover potential drug targets in this high-risk group by comparing global expression profiles of leukemia-initiating cells from patients with AML who have since deceased and those of normal human HSPCs (Fig. 1b). We identified leukemia-initiating populations by determining in vivo human AML engraftment (CD45+CD33+ AML engraftment without T or B cell engraftment) in multiple NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ (NSG) newborn mice (flow cytometry gating strategy, representative flow cytometry plots and morphology of engrafted cells are shown in Supplementary Figs. 1 and 2). We performed RNA-seq on leukemia-engrafting cells identified from 86 patients (patients with FLT3 WT, n = 54; patients with mutated FLT3, n = 32) and on cord blood (CB) CD34+ HSPCs obtained from healthy donors (n = 44). In FLT3 WT leukemia-engrafting cells, 3,616 differentially expressed genes (DEGs) were identified (absolute log₂ (fold change (FC)) > 1, adjusted P value < 0.05; principal component analysis (PCA) plot in Fig. 1c, volcano plot in Extended Data Fig. 1b; 2,904 upregulated genes, log₂ (FC) > 1, adjusted P value < 0.05; 712 downregulated genes, log₂ (FC) < -1, adjusted P value < 0.05). In FLT3-mutated leukemia-engrafting cells, 4,346 DEGs were identified (absolute log₂ (FC) > 1, adjusted P value < 0.05; PCA plot in Fig. 1c, volcano plot in Extended Data Fig. 2c; 3,216 upregulated genes, log₂ (FC) > 1, adjusted P value < 0.05; 1,130 downregulated genes, log₂ (FC) < -1, adjusted P value < 0.05). To identify pathways with pathophysiological relevance, we focused on 1,284 DEGs upregulated both in FLT3 WT and Articles FLT3-mutated AML samples (log₂ (FC) > 1.5, adjusted P value < 0.05, base mean > 20 reads per kb of transcript per million reads mapped (RPKM)) (Fig. 1d). In AML cells, 325 Gene Ontology (GO) terms were enriched (GORilla^{19,20}; exact P value < 0.001), revealing a cluster of GO terms related to the regulation of apoptotic process and cell proliferation (REVIGO²¹; Fig. 1e,f). Protein–protein interaction analysis of the 1,284 DEGs showed subnetworks of interrelated proteins (STRING²²; Fig. 1g, left), with prominent nodes involving the mitotic

cell cycle (that is, cyclin-dependent kinase (CDK)1, cell division cycle (CDC)20, cyclin (CCN)B1, CCNB2, kinesin family member (KIF)20A, non-SMC condensin I complex subunit G (NCAPG)) and regulators of apoptosis, including baculoviral IAP repeat (BIR)-containing (BIRC)5, a member of IAP gene family (Fig. 1g, right, and Fig. 1h). These findings identify potential dependencies in leukemia-engrafting cells from highly aggressive, poor-prognosis AML. Treatment-resistant FLT3 WT and FLT3-ITD+ AML demonstrate dependencies on distinct survival- and proliferation-related pathways. To examine whether these potential dependencies could be therapeutically exploited, we screened 35 available small-molecule inhibitors targeting products of upregulated DEGs and enriched pathways against primary relapsed or refractory FLT3 WT (n = 8) and FLT3-ITD+ (n = 2) AML (Supplementary Tables 2 and 3, patients 1–10). AML cell elimination, quantified as a fraction of the number of viable cells compared with that in the vehicle control following a 72-h in vitro exposure, is shown as a heatmap (Fig. 2a; representative data from patients 1 and 2 are shown in Extended Data Fig. 1d). Of the 35 compounds, eight showed greater than 70% killing of AML cells at 300 nM in at least six of ten patients tested (AZD5582, YM155, venetoclax, S63845, dinaciclib, GSK923295, SB743921, barasertib). Among them, AZD5582 exhibited the greatest efficacy and potency, with greater than 70% killing achieved in nine of ten samples from patients with AML at 300 nM and seven of ten samples at 10 nM. Identification and characterization of AZD5582, a bivalent SMAC-mimetic IAP antagonist, were previously reported¹⁵. XIAP (BIRC4), an IAP family member, directly binds to and sequesters proapoptotic caspases through its BIR2 and BIR3 domains, exerting its anti-apoptotic function. Specifically, the BIR3 domain of XIAP inhibits the catalytic activity of caspase-9 by preventing its homodimerization²³, while the BIR2 domain binding to caspase-3 and caspase-7 blocks their catalytic sites^{24,25}. AZD5582 potently binds and inhibits both BIR2 and BIR3 domains of BIRC4 (XIAP) and inhibits the BIR3 domain of another IAP family member, cellular (c)IAP1 (BIRC2), which plays a complementary role in apoptotic signaling through ubiquitination of key proteins involved in canonical and noncanonical nuclear factor (NF)- κ B signaling^{26,27}. AZD5582, active against only 34 of 200 cancer cell lines, was not broadly cytotoxic¹⁵. Extensive pharmacodynamics and safety maintained in the presence of AZD5582 (Extended Data Fig. 1g), while AZD5582 showed greater effects against AML cells than native T cells in patient samples (viable CD33+ human AML cells, 9.3% \pm 1.8%; viable CD3+ human T cells, 77.2% \pm 6.9%; n = 15 cases, P = 5.272 \times 10⁻⁷ by paired two-tailed t-test) (Extended Data Fig. 1h). Next, we performed a validation and extension in vitro screen using five compounds (AZD5582 at 10 nM, 30 nM and 100 nM for FLT3-ITD+ samples and at 30 nM and 100 nM for FLT3 WT samples; venetoclax, S63845, barasertib and GSK923295 each at 100 nM and 300 nM) against a cohort of 59 high-risk AML samples (FLT3 WT, n = 43; FLT3-ITD+, n = 16) (Fig. 2b and Supplementary Table 2, patients 1–59). These compounds inhibit distinct pathways: XIAP/cIAP1 (AZD5582), BCL2 (venetoclax), MCL1 (S63845), AURKB (barasertib) and centromere protein (CENP)E (GSK923295). We deemed a patient sample to be responsive or sensitive (and the compound to be efficacious against the patient sample) if the compound achieved greater than or equal to 70% elimination of AML cells at 30 nM for AZD5582, 300 nM for venetoclax and S63845 and 100 nM for barasertib and GSK923295. Conversely, a patient sample was deemed resistant to a compound if less than 70% elimination of AML cells could be achieved. AZD5582 was effective against 30 of 43 FLT3 WT patient samples (cases) (69.8%), while venetoclax, S63845, barasertib and GSK923295 showed efficacy against 20, 9, 11 and 4 of 43 cases (46.5%, 20.9%, 25.6% and 9.3%), respectively. AZD5582 was more frequently effective against cases with adverse-risk

karyotypes¹⁶: 23 of 32 samples with complex karyotype, 20 of 26 samples involving chromosome 5, 7 and/or 17 abnormalities, four of four samples with t(3;3) (q21;q26.2) or inv(3) (q21q26) and four of four samples with the t(v;11q23.3) KMT2A (MLL) translocation; 35 of 43 samples with adverse-risk karyotypes overall (Fig. 2b,c). Surprisingly, although AZD5582 does not directly target normal or mutated FLT3, 14 of 16 FLT3-ITD+ cases (87.5%) showed responsiveness to AZD5582. In addition, at low concentrations, AZD5582 was more effective against FLT3-ITD+ samples than were FLT3 inhibitors quizartinib, crenolanib, gilteritinib and midostaurin (Fig. 2d). Moreover, AZD5582 demonstrated efficacy against five of six poor-prognosis imatinib-resistant Ph+ acute lymphoblastic leukemia and MPAL cases, although BCR–ABL (breakpoint cluster region–Abelson proto-oncogene) kinase is not directly targeted (Fig. 2e and Supplementary Table 3, patients 60–65). Venetoclax (four of six cases) and S63845 (two of six cases) were also effective, whereas barasertib and GSK923295 were ineffective. These findings further demonstrate that AZD5582 was effective in the highest frequency of patients examined with high-risk AML. A number of previous studies showed that IAP-binding motif-bearing SMAC mimetics induce ubiquitination and degradation of XIAP and cIAP1 in cell lines^{26,27,29–31}. AZD5582 exposure led to downregulation of XIAP and induction of activated caspase-3 in AZD5582-sensitive AML cells but not in AZD5582-resistant Articles AML cells, strongly suggesting that AZD5582 exerts its cytotoxicity through degradation of XIAP (Fig. 2f). Moreover, guide (g) RNA-mediated targeting of XIAP (BIRC4) and cIAP1 (BIRC2) reduced viability of AZD5582-sensitive Molm13 cells but not that of AZD5582-resistant TF1a cells (Extended Data Fig. 2a). While AZD5582 was more potent than birinapant (a bivalent IAP inhibitor) and AT406 (a monovalent IAP inhibitor), there was a correlation between response profiles of AZD5582 and birinapant (Extended Data Fig. 2b–d; Pearson’s correlation test, $r = 0.9864$, two-tailed $P = 0.0001$). On the other hand, response profiles of venetoclax, S63845, barasertib and GSK923295 did not correlate with that of AZD5582, indicating the effectiveness of bivalent SMAC-mimetic IAP antagonists as a class. AZD5582 responsiveness is modulated by AML-associated somatic mutations and aberrant chromosomes. To identify determinants of responsiveness to AZD5582 and the other four compounds, we next examined the correlation between in vitro leukemia cell elimination and mutational or karyotypic landscapes. For 107 AML cases, we performed targeted DNA sequencing for 41 AML-associated somatic mutations (Supplementary Table 5). AZD5582 showed efficacy across samples with diverse somatic mutations and chromosomal abnormalities, with particular efficacy against cases with mutated NRAS, FLT3, NPM1, IDH2, TET2, GATA2 and CBL (Fig. 3a) and abnormalities involving chromosome 7 (–7), chromosome 3 (inv(3), t(3;3), other translocations) and MLL rearrangements (Fig. 3b). Correlations between mutations or cytogenetics and sensitivity or resistance to the five compounds were identified, including that of venetoclax responsiveness with mutations in IDH1 and IDH2, consistent with previous reports^{32,33}, that of TP53 and IDH1 mutations with AZD5582 resistance and that of TP53 and CBL mutations with venetoclax resistance (Fig. 3c,d and Supplementary Tables 6 and 7). While TP53 mutations correlated with resistance to both AZD5582 and venetoclax, they did not determine responsiveness to these drugs completely, as 13 of 73 AZD5582-sensitive cases and 11 of 61 venetoclax-sensitive cases had TP53 mutations. In vitro responses of 107 AML cases with diverse mutations in order of AZD5582 responsiveness (Fig. 4a, top) and in order of venetoclax responsiveness (Fig. 4a, bottom, $n = 103$ AML cases) are shown. Seventy-three of 107 (68.2%) cases were responsive to AZD5582 and 61 of 103 (59.2%) were responsive to venetoclax, while S63845, barasertib and GSK923295 each

showed efficacy in 23 of 66 (34.8%), 21 of 66 (31.8%) and 11 of 66 (16.7%) AML cases (data shown in Extended Data Fig. 3). While these findings demonstrate that XIAP dependence is an important mechanism for survival of highly aggressive AML, no single somatic mutation or chromosomal abnormality completely defined responsiveness to these molecular targeted drugs. Therefore, we endeavored to define AZD5582 and venetoclax responsiveness through a combinatorial FLT3 and WT IDH1 and IDH2 were sensitive to AZD5582, suggesting that mutated IDH1 and/or IDH2 diminishes XIAP dependence in leukemia cells with mutated FLT3. In addition, three of 14 cases with mutated NRAS carried complex karyotypes and TP53, IDH1 or RUNX1 mutations and were resistant to AZD5582. Similarly, one of nine cases with mutated CBL had mutated TP53 and RUNX1 and was resistant to AZD5582. These findings suggest that XIAP dependence is conferred by mutated FLT3, NRAS and CBL but is moderated by TP53, IDH1, IDH2 and RUNX1 mutations and complex karyotype. Importantly, nine cases with resistance to both AZD5582 and venetoclax carried complex karyotype ($n = 7$) and mutated TP53 ($n = 7$), with six cases having both. Moreover, six cases with both complex karyotype and mutated TP53 were resistant to all five compounds, showing that these two genetic abnormalities contributed to diminished dependence on multiple survival pathways in highly aggressive AML. Dependence of leukemic cells on XIAP for survival positively correlates with TP53 transcriptional activity, while EVI1 activation leads to resistance to BCL2 inhibition. These findings suggest that aberrant TP53 contributes to diminished dependence on multiple survival pathways in highly aggressive AML. However, we found that the presence of mutated TP53 alone was not sufficient to predict sensitivity or resistance to XIAP inhibition. Among 29 cases with mutated TP53, 14 were responsive to AZD5582, and 15 were resistant to AZD5582, reflecting the heterogeneity of mutations themselves and their effects on downstream function. To link TP53 mutation and the resultant disruption in TP53 function with XIAP dependence, we used motif activity response analysis. Motif activity quantifies the regulatory function of a transcription factor based on the RNA-seq expression level of genes with predicted DNA-binding site motifs for the transcription factor in their promoter region^{34,35}. Focusing on samples with mutated TP53, preservation of TP53 motif activity and AZD5582 responsiveness were positively correlated (Pearson's correlation coefficient $r = 0.5063$, two-sided $P = 0.0071$) (Fig. 5a). Furthermore, TP53 mutations predicted to have more deleterious consequences on TP53 function (for example, frameshift mutations and deletions) were associated with AZD5582 resistance (Fig. 5b). Moreover, mutations near functional domains of the TP53 protein (for example, Glu286Val near the DNA-binding surface and His179Tyr near the zinc-finger domain) were associated with diminished TP53 motif activity and diminished AZD5582 responsiveness, while mutations distant from functional domains (for example, Tyr220Cys) was associated with preserved TP53 motif activity and AZD5582 responsiveness (Fig. 5c and Supplementary Videos 1 and 2). These findings suggest that TP53 motif activity may inform AZD5582 responsiveness in patients with AML and TP53 mutations. To explore mechanisms for XIAP dependence in AML, we identified 23 TP53 target genes (with TP53-binding motifs in their promoter regions) that were differentially expressed between AZD5582-sensitive AML cells with high TP53 motif activity and AZD5582-resistant AML cells with low TP53 motif activity (Fig. 5d). Among the DEGs, we found two putative TP53-regulated genes reported to bind TP53 by chromatin immunoprecipitation sequencing analysis in Molm13 and K562 cell lines³⁶: BCL2 binding component 3 (BBC3) and apoptosis-enhancing nuclease (AEN). BBC3 encodes PUMA, a BH3-only proapoptotic protein, which interacts with anti-apoptotic BCL2 family members,

resulting in release of mitochondrial apoptogenic proteins, including SMAC^{37,38}. AEN is an exonuclease with apoptosis-inducing ability and is required for efficient DNA fragmentation in TP53-dependent apoptosis³⁹. To directly demonstrate transcriptional regulation of BBC3 and AEN by TP53, we performed cap analysis gene expression (CAGE) analysis in primary human AML cells. CAGE is a powerful tool that identifies transcription start sites by detecting the 5' end of NATURE CANCER cDNA through the cap capture method and allows assessment of promoter activity for each transcript^{40,41}. We found a dominantly active promoter containing a TP53-binding motif upstream of one of the four BBC3 transcripts (ENST00000439096.2) in AML cells (Fig. 5e and Extended Data Fig. 4). Activity of this BBC3 promoter was suppressed in AZD5582-resistant cells carrying TP53 mutations associated with reduced TP53 motif activity, such as Ile255del, Glu286Val and Val73fs (Fig. 5b and Extended Data Fig. 4b), while mutants with high motif activity (for example, TP53 Arg282Trp and Tyr220Cys) and AZD5582 responsiveness demonstrated substantial specific binding to the BBC3 promoter (Fig. 5b and Extended Data Fig. 4c,d). Similarly, transcriptional activity from the AEN promoter containing the TP53-binding motif was suppressed in AZD5582-resistant AML cells with reduced TP53 motif activity (Extended Data Fig. 4e–h). These findings indicate that preserved TP53-regulated expression of BBC3 and AEN contributes to XIAP dependence in poor-prognosis AML. Next, we explored potential mechanisms underlying the distinct dependencies on XIAP and BCL2 in AZD5582-sensitive venetoclax-resistant t(3;3) (q21;q26) and inv(3q21) AML cells (Fig. 3d and Supplementary Table 7). Chromosomal abnormalities involving 3q21 result in activation of EVI1 (MECOM) transcription by the GATA2 enhancer^{42,43}. Indeed, we found high levels of EVI1 (MECOM) expression in AML cells with t(3;3) (q21;q26) and inv(3q21) (green and orange circles, Fig. 5f; $P = 9.936 \times 10^{-6}$ by unpaired two-tailed t-test). Moreover, GATA motif activity was low in AML cells with t(3;3) and inv(3) (green and orange circles, Fig. 5g; $P = 0.0057$ by unpaired two-tailed t-test), implying an association of reduced transcriptional activation of GATA-target genes with BCL2 independence. In the clinical setting, timely evaluation of drugs is required for rapid initiation of treatment. Therefore, we examined the utility of a short time course drug exposure followed by flow cytometric evaluation of apoptosis and cell death by measuring Annexin V expression and staining with 7-aminoactinomycin (7-AAD), respectively. In AZD5582-sensitive samples ($n = 9$), the frequency of 7-AAD–Annexin V+ apoptotic cells increased significantly at 4 h and 6 h post-exposure ($P = 0.01$ and $P = 6.43 \times 10^{-4}$, respectively, by paired two-tailed t-tests compared with that at 2 h post-exposure), reaching above 10% at 6 h (Fig. 5h, left). Concordantly, the increased frequency of 7-AAD+Annexin V+ dead cells started at 6 h post-exposure ($P = 0.0463$ by paired two-tailed t-test compared with that at 2 h post-exposure), exceeding 20% at 24 h post-exposure ($P = 0.000167$ by paired two-tailed t-test compared with that at 2 h post-exposure). Conversely, in samples that were relatively resistant to AZD5582 ($n = 6$), frequencies of apoptotic cells at 6 h and dead cells at 24 h post-exposure remained less than 5% and 10%, respectively (Fig. 5h, right). Thus, a rapid 6-h in vitro exposure followed by flow cytometric measurement of Annexin V can predict AZD5582 responsiveness. This substantially shortens the time from clinical presentation to determination of drug sensitivity, allowing for identification of patient-specific optimized combination drug regimen in a clinically feasible timescale. Effective in vivo treatment choices as informed by in vitro treatment efficacy and genetic information. Through in vitro screening, we established an efficacy matrix of AZD5582 (XIAP inhibition), venetoclax (BCL2 inhibition), S63845 (MCL1 inhibition), barasertib (AURKB inhibition) and GSK923295 (CENPE inhibition) against high-risk poor-outcome AML. Figure 6

presents in vitro single-agent responses of patient samples to the five compounds in pairs, showing the distribution of patient samples that were responsive to multiple compounds in vitro. In our patient cohort, AZD5582 and venetoclax were most frequently effective in vitro (Fig. 6a, leftmost panel). Of note, responsiveness to barasertib and GSK923295 were highly correlated (Fig. 6d).

These findings suggest that optimized drug combination against highly aggressive AML can be informed by patterns of in vitro responsiveness as well as by genetic events including somatic mutations, chromosome abnormalities and altered gene regulatory networks inherent to each patient. To validate this hypothesis, we created PDX models of 22 high-risk poor-outcome AML cases for in vivo therapeutic testing. The 22 cases reflected diverse genetic profiles in our cohort and were primarily refractory or relapsed, with 21 patients succumbing to the disease (one patient who relapsed was lost to follow-up) (clinical information is provided in Supplementary Table 3). For each PDX model, AML engraftment was confirmed in the PB, first at 6 weeks post-transplantation and then every 3 weeks thereafter; human AML chimerism in PB was assessed weekly during in vivo treatment and in the BM and spleen at the end of a 4-week treatment course (Supplementary Table 8). For AZD5582-responsive venetoclax-responsive cases, we examined the in vivo effect of each compound as a single agent and in combination (Fig. 7a and Extended Data Fig. 5a). For patient 9 (FLT3-ITD+, mutated DNMT3A, NPM1 and PPM1D, complex karyotype), we found that AZD5582 alone completely eliminated AML cells in the BM, spleen and PB. In patient 1 (FLT3 WT, mutated CBL, NRAS, IDH2, TET2, DNMT3A, NPM1 and SETBP1, complex karyotype), single-agent AZD5582 and venetoclax eliminated AML cells in some but not all recipients. However, AZD5582–venetoclax combination treatment was highly efficacious. In vivo, AZD5582venetoclax showed similar efficacy in samples from patients 53 (FLT3-ITD+ and mutated WT1, monosomy 7, complex karyotype), 33 (FLT3-TKD+, mutated TET2, WT1 and SMC3, MLL-MLLT1 (ENL)), 22 (FLT3 WT, mutated TP53, GATA2 and monoallelic CEBPA, chromosome 17 abnormality, trisomy 21, monosomy 7, complex karyotype) and 2 (mutated monoallelic CEBPA, PPM1D, trisomy 21), validating in vitro responsiveness. In addition, the AZD5582–S63845 combination showed efficacy in samples from patients 1 (FLT3 WT, mutated CBL, NRAS, IDH2, TET2, DNMT3A, NPM1 and SETBP1, complex karyotype), 47 (FLT3-ITD+, mutated DNMT3A, NPM1, TET2, monoallelic CEBPA, ASXL2, CUX1, NF1, ZRSR2) and 34 (FLT3-TKD+, mutated NF1, monosomy 7, monosomy 17, complex karyotype), validating in vitro responsiveness to XIAP and MCL1 inhibition. Among AZD5582-sensitive, venetoclax-resistant samples, those from patient 48 (FLT3-ITD+, mutated TET2, MGA, complex karyotype), with high levels of sensitivity to AZD5582 in vitro, demonstrated responsiveness in vivo (Fig. 7b and Extended Data Fig. 5a). Samples from patients 51 (FLT3-ITD+, mutated KMT2A, t(3;3) (q21;q26.2) and monosomy 7) and 4 (FLT3 WT, mutated NRAS, DNMT3A, BCOR, complex karyotype) showed responsiveness to the AZD5582–barasertib combination in vivo, validating in vitro findings. Samples from patient 52 (FLT3-ITD+, mutant TP53, DNMT3A, NPM1, monoallelic CEBPA and ETV6), showed responsiveness to the AZD5582–S63845 combination in vivo, validating in vitro sensitivity to XIAP and MCL1 inhibition. Lastly, among AZD5582-resistant AML samples, those from patient 7 (mutated PTPN11, MGA, PHF6), showed responsiveness to venetoclax in vivo, validating high levels of sensitivity in vitro (Fig. 7c and Extended Data Fig. 5a). In samples from patient 58 (FLT3-ITD+, mutated IDH1, NPM1, DNMT3A), the effective venetoclax–S63845 combination in vivo confirmed in vitro findings. Interestingly, AML cells from this patient with FLT3-ITD+ were resistant to the FLT3 inhibitor quizartinib in vivo, indicating that in vitro sensitivity

assessment can help discover effective drug combinations beyond currently available targeted agents. In each case, normal murine hematopoiesis recovered concurrently with leukemia clearance during AZD5582-based combination treatment. At the end of the 4-week treatment course, murine trilineage hematopoiesis (erythropoiesis, megakaryopoiesis and myelopoiesis) were detected in situ in the BM and spleen (Fig. 5b,c, larger images in Extended Data Fig. 6a). Elimination of AML cells was accompanied by the recovery of mouse CD45+ hematopoietic cells, including Mac1+Gr1⁻ monocytes and Mac1+Gr1⁺ granulocytes in the PB (Extended Data Fig. 6b,c). Efficacies of AZD5582-based combinations are summarized in Fig. 8a (individual PDX model mouse data are summarized in Supplementary Table 8 and Extended Data Figs. 7 and 8). Finally, PDX models created from five patients (1, 9, 10, 22, 53) underwent a 4-week course of AZD5582–venetoclax in vivo followed by over 4 weeks of observation off-treatment. In these mice, we found no evidence of AML relapse in the BM, spleen or PB (Fig. 8b). There was transient weight loss (mean, less than 5%) with no changes in serum chemistries, indicating that long-term AZD5582–venetoclax combination treatment did not cause severe generalized toxicity (Supplementary Tables 9 and 10). Again, we confirmed concurrent leukemia clearance and murine trilineage hematopoietic recovery in the BM (Extended Data Fig. 9a,b).

Discussion Over the past decade, diversity in somatic mutations in patients with AML have become clear through DNA sequencing^{44,45}. This genetic complexity and heterogeneity complicate targeted drug treatment strategies for AML. Targeted drugs with more favorable toxicity profiles are now viable options for patients with gain-of-function mutations, such as FLT3-ITD⁺, IDH1 and IDH2, especially for the elderly with comorbidities or those failing multiple rounds of conventional therapies^{2,3,46}. However, the long-term outcome for relapsed and refractory patients continues to be poor⁴⁷. Based on this background, the aim of this study was to develop effective therapeutic strategies for high-risk AML, focusing on a cohort not only presented with adverse prognosis but also demonstrating poor clinical outcomes, for example, primary refractoriness, post-chemotherapy or post-hematopoietic stem cell transplantation relapse and ultimately succumbing to the disease. This group of high-risk poor-outcome patients was associated with multiple somatic mutations and complex karyotypic abnormalities. To identify a strategy to eradicate AML cells in such patients, our approach was to target critical pathways and regulatory networks rather than individual abnormal proteins resulting from gene mutations or chromosomal abnormalities. Inhibition of five interrelating pathways led to effective elimination of AML cells despite substantial heterogeneity in genetic events, identifying patient-specific NATURE CANCER profiles of vulnerabilities. Surprisingly, maximal vulnerability for these genetically and biologically diverse patient-derived AML cells converged to the inhibition of two anti-apoptotic proteins: XIAP and BCL2. In over 90% of cases failing conventional chemotherapy and/or hematopoietic stem cell transplantation, we uncovered dependence on these proteins for survival as evidenced by effective targeting of AML cells. In vivo combined treatment based on this patient-specific responsiveness profile resulted in complete elimination of AML cells, even in the NSG PDX environment lacking human anti-tumor immune cells. At the same time, XIAP inhibition by AZD5582 did not significantly impair in vitro proliferation and differentiation capacity of human CB HSPCs nor affect normal murine hematopoiesis substantially in vivo (Supplementary Table 11), consistent with previous report demonstrating limited off-target effects and lack of generalized toxicity or activation of the immune system in vivo in mice and in macaques²⁸. Importantly, an in vitro sensitivity assessment could be performed in as little as 6 h by assessing apoptotic cell death of AML cells by flow cytometry, allowing

determination of patient-specific optimized combination drug regimen in a clinically feasible timescale using methodology already widely available in clinical laboratories. Determinants of drug responsiveness in high-risk AML with complex genetic characteristics included (1) mutated FLT3, either ITD or TKD, conferred high responsiveness to both AZD5582 and venetoclax, (2) concurrent IDH1 mutation diminished dependence on XIAP while increasing vulnerability to BCL2 inhibition in FLT3-mutated AML, and (3) mutated CBL was associated with AZD5582 responsiveness and venetoclax resistance (Supplementary Table 12). Efficacy of AZD5582, an IAP inhibitor, in cases with mutations in specific kinases, such as FLT3, suggests its potential effectiveness against malignant cells with other mutated kinases. Potent vulnerability to XIAP inhibition was also found in the setting of chromosomal aberrations, including clinically aggressive AML with MLL-AF9 (KMT2A-MLLT3) MLL-MLLT1-AF9, MLL-ENL (KMT2A-MLLT1) MLL-MLLT3-ENL and MLL-AF6 (KMT2A-AFDN) MLL-AFDN-AF6 translocations and concurrent mutations such as FLT3, IDH2 and GATA2, extending previously reported findings in a genetically engineered mouse model of MLL-MLLT1-AF9 leukemia⁴⁸. Monosomy 7 highly correlated with venetoclax resistance; loss of the gene encoding cytochrome C may contribute to reduced BCL2 dependence in these cases (Extended Data Fig. 10a). Interestingly, a recent study reported that monocytic AML is resistant to BCL2 inhibition but is instead dependent on MCL1⁴⁹. In our cohort, venetoclax-resistant S63845-responsive cases were responsive to AZD5582, suggesting that combined XIAP-MCL1 inhibition may be effective in these cases. Complex genetic events certainly underlie treatment resistance and poor clinical outcome in AML, and we identified a set of criteria that help predict the optimal treatment for over 80% (85 of 103) of our cohort: (1) somatic mutations in six recurrently mutated genes (FLT3, IDH1, IDH2, CBL, NRAS and TET2), (2) abnormalities involving two chromosomes (translocation and inversion involving 3q21 and translocation involving chromosome 11q23) and (3) TP53 motif activity in cases with TP53 mutations. In particular, TP53 motif activity positively correlated with XIAP dependence in cases with TP53 mutations regardless of concurrent somatic mutations or karyotype (Extended Data Fig. 10b). In the remaining 18 of 103 cases, highly heterogeneous combinations of somatic mutations and chromosomal abnormalities were present. The genetic mechanisms determining dependence on XIAP and/or BCL2 for survival in these cases will await future study. Correlations of genetic abnormalities and dependence on XIAP or BCL2 anti-apoptotic pathways based on in vitro responses to AZD5582 and venetoclax in 103 high-risk poor-outcome AML samples are summarized in Extended Data Fig. 10c. AML with mutated TP53 is associated with particularly poor outcomes, making identification of effective therapeutic options essential. While mutated TP53 alone was not sufficient to predict XIAP-dependent phenotype, preserved TP53 transcriptional regulatory function, as determined by TP53 motif activity, correlated with XIAP dependence. However, determination of TP53 motif activity requires RNA-seq. Identifying a limited number of TP53-regulated genes as surrogates simply measured by targeted PCR would facilitate clinical implementation; BBC3 and AEN were identified as such surrogates. BBC3, otherwise known as PUMA, is a BH3-only protein exerting its proapoptotic function by relieving the inhibition of anti-apoptotic BCL2 family proteins, resulting in the release of mitochondrial apoptogenic proteins including SMAC^{37,38}. Our findings are consistent with previous reports showing TP53-dependent transcriptional activation of BBC3 and BBC3-induced release of apoptogenic proteins, including SMAC in primary AML cells, activated CD4+ T cells and a variety of malignant human AML and solid tumor cell lines^{50–53}. In high-risk AML cells with aberrant TP53 transcriptional regulatory function and reduced TP53-mediated

BBC3 transcription, diminished SMAC release and inhibited degradation of XIAP and other IAPs may lead to resistance to IAP inhibitors. Identification of treatment response-related TP53-regulated genes and their function in treatment resistance awaits future study. Two other mechanisms implicated in attenuating XIAP inhibition include its degradation through activated AKT and Notch pathways. In AZD5582-resistant pancreatic cancer cell lines expressing phosphorylated AKT, AKT knockdown reversed AZD5582 resistance⁵⁴. The transactivation domain of Notch was shown to bind directly to the RING domain of XIAP and inhibit XIAP ubiquitination and degradation, leading to suppression of apoptosis in the Jurkat T cell leukemia cell line⁵⁵. Overall, 94 of 103 (91.3%) high-risk poor-outcome cases showed high responsiveness to AZD5582 and/or venetoclax. Integrative analysis of somatic mutations, chromosomal abnormalities, gene expression and regulatory networks in diversified international patient populations will await future study. Through such efforts, we expect that a patient-specific, precision-medicine approach for adverse-risk poor-outcome leukemia will become possible. Understanding the biology of stem cells in leukemia has paved a path for the study of other malignancies^{56–58}. Similarly, identifying vulnerabilities in highly aggressive leukemia will lead to the development of effective treatment strategies for other malignancies.

Methods

Human samples. All patient samples were obtained from Toranomon Hospital under written informed consent. The study was performed with authorization from the Institutional Review Board for Human Research at RIKEN and Toranomon Hospital, in accordance with the ethical standards of responsible committees on human experimentation at each institution. CB cells were obtained from the Chubu Cord Blood Bank. Mice and xenogeneic transplantation. Immune-compromised NSG mice were bred and maintained with defined flora at the animal facility of RIKEN and at Jackson Laboratory, conditioned at 23 ± 2 °C in $50\% \pm 10\%$ humidity, on a 12-h dark–light cycle. All experiments were performed with authorization from and according to guidelines established by the Institutional Animal Committees at RIKEN and Jackson Laboratory. Both female and male newborn NSG mice received 1.5 Gy total body irradiation followed by intravenous injection of sorted human cells within 72 h of birth. The extent of engraftment of human cells in the NSG recipients was assessed by retro-orbital phlebotomy and flow cytometry. Treatment studies were conducted when sufficient engraftment was observed, at approximately 6 weeks of age. Flow cytometry. The list of monoclonal antibodies used is included in the Nature Research Reporting Summary. Analyses were performed with the FACS Aria III and FACSCanto II (BD). RNA-seq and bioinformatic analyses. RNA extraction and RNA-seq analysis were performed as described previously⁶¹. Briefly, RNA was extracted using TRIzol reagent (1559601, Invitrogen) from original patient samples or from hCD45+ cells isolated from BM cells of recipient mice. The NEBNext Ultra RNA Library Prep kit for Illumina (E7530, New England Biolabs) was used for RNA library preparation. Articles Final library size distribution was validated using a Bioanalyzer (Agilent) and quantified using quantitative PCR. The DNA libraries were hybridized to a flow cell, amplified on the Illumina cBot and subsequently run on the HiSeq 2500 (Illumina, using 50-base single-end read mode). The sequence reads were mapped to the human genome (NCBI version 19) using TopHat2 version 2.0.8 and Bowtie2 version 2.1.0 with default parameters, and gene annotation was provided by NCBI RefSeq. The transcript abundances were estimated using Cufflinks (version 2.1.1). DESeq2 version 1.22.2 (ref. 62) was used for differential gene expression analysis. We required a Benjamini–Hochberg adjusted P value less than 0.05 and absolute log₂ (FC) greater than 1.0 to identify DEGs. The programming framework R version 3.5.3 was used to call DEGs. GO term enrichment analysis was performed using GOrilla^{19,20}

and REVIGO21. Target genome sequencing. Target genome sequencing was performed on DNA extracted from original patient samples or hCD45+ cells isolated from BM from the PDX model. DNA extraction was conducted using the DNeasy Blood and Tissue kit (Qiagen). Using DNA, we analyzed all coding regions and 2-bp flanking intronic sequences of the 41 genes established as related to AML. The total length of the target region was 127,151 bp. A two-step PCR method was used to construct DNA libraries according to previously published methods^{63,64}. After purification and quantification, pooled libraries were sequenced by 2 × 150-bp paired-end reads on the HiSeq 2500 (Illumina). ClinVar⁶⁵ was also used to discriminate pathogenic mutations from mutations that were likely benign. Cap analysis gene expression. CAGE, a quantitative genome-wide assay of transcription start sites at single-base-pair resolution, was performed using a modification of a previously published protocol⁶⁶. The detailed protocol can be found at <https://www.protocols.io/view/low-quantity-single-strand-cage-protocol-bbwkipcw>. Compounds. The compounds used in this study are listed in Supplementary Table 2. In vitro chemical screening. The in vitro viability assay was performed as previously described⁶⁷. In short, patient-derived leukemia cells were seeded at 8×10^4 – 1×10^5 cells per well in Stemline II Hematopoietic Stem Cell Expansion Medium supplemented with stem cell factor (50 ng ml⁻¹), FLT3 ligand (50 ng ml⁻¹) and thrombopoietin (50 ng ml⁻¹) in 96-well plates. For in vitro evaluation of T cells in addition to AML cells in patient samples, interleukin (IL)-2 (5 ng ml⁻¹), IL-3 (20 ng ml⁻¹), IL-7 (20 ng ml⁻¹) and IL-15 (10 ng ml⁻¹) were also added. Cells were exposed to small molecules at the indicated concentrations for 72 h at 37 °C in a humidified atmosphere containing 5% CO₂. Cells were collected and stained with BV421-labeled anti-hCD45 antibody and 7-AAD, collected in BD Trucount Tubes (340334, BD) and analyzed using the FACSCanto II (BD). In vivo treatment. In vivo treatment experiments were performed with AML-engrafted NSG recipients using the compounds listed in Supplementary Table 2. The recipients were treated with AZD5582 (Selleck) (1 mg per kg) intraperitoneally once a day, venetoclax (ABT-199, Active Biochem) (70 mg per kg) orally once a day, S63845 (Selleck) (25 mg per kg), barasertib (Selleck) (25 mg per kg), GSK293295 (Selleck) (50 mg per kg) or quizartinib (Biomol) (10 mg per kg). For combination treatment, doses of AZD5582 and venetoclax were halved. The mice were euthanized when they became moribund or after 4–6 weeks of treatment. Human AML chimerism in the BM, spleen and PB was determined using flow cytometry. All treated recipients and their pre- and post-treatment engraftment data are tabulated in Supplementary Table 8. For AZD5582 treatment in unirradiated non-transplanted NSG mice, AZD5582 (Selleck) (1 mg per kg) was administered intraperitoneally once a day for 2 weeks, and BM and PB were collected after treatment. Total white blood cell counts, hemoglobin concentrations and platelet counts were obtained using an automated hematology analyzer (XT-2001i, Sysmex), and frequencies of Mac1+Gr⁻ monocytes and Mac1+Gr⁺ granulocytes were determined by flow cytometry. Morphological analysis. Cytospin specimens were prepared using a Shandon Cytospin 4 cytocentrifuge (Thermo Electric). May–Grünwald–Giemsa staining was performed using standard procedures. Light microscopy was performed using the Zeiss Axiovert 200 (Zeiss). Immunoblotting. BM cells isolated from mice with PDXs were incubated in 250 µl culture medium with vehicle (dimethylsulfoxide) or 30 nM AZD5582. Whole cell lysates were prepared from 1×10^6 cells using 100 µl modified radioimmunoprecipitation assay buffer (Fujifilm) supplemented with 1% protease inhibitor cocktail (Merck) and were stored at –80 °C until testing. Protein concentrations were analyzed using the BCA protein assay kit (Thermo Fisher Scientific) following the

manufacturer's instructions. For immunoblotting, 10 or 15 µg of whole cell lysates were mixed with Laemmli sample buffer and heated at 95 °C for 5 min. The samples were loaded on a gel for SDS-PAGE using a SuperSep Ace 15% gel (Wako). The proteins were transferred to a nitrocellulose membrane (Bio-Rad), blocked with 5% skim milk in 0.1% Tween-20 for 1 h at room temperature and then incubated overnight with anti-XIAP antibody (1:1,000 dilution, 2045S, CST) or anti-cleaved caspase-3 antibody (1:1,000 dilution, ab32042, Abcam). The membrane was treated with secondary antibody at room temperature for 1 h. Protein detection was conducted using enhanced chemiluminescence reagents (Thermo Fisher Scientific) or ECL Prime Western Blotting Detection Reagent (GE Healthcare) on the ATTO LuminoGraph 3 system (ATTO). Staining with anti-GAPDH antibody (1:1,000 dilution, sc-32233, Santa Cruz) was performed as a loading control. Unprocessed images are provided in the source data.

Colony-forming cell assay. CB-derived CD34+ cells were exposed to AZD5582 at 30 nM or vehicle in Stemline II medium (Merck) supplemented with stem cell factor (50 ng ml⁻¹), FLT3 ligand (50 ng ml⁻¹) and thrombopoietin (50 ng ml⁻¹) in 96-well plates. After 3 d, single CB CD34+CD38- cells were sorted and plated into wells of 96-well plates containing 150 µl complete MethoCult medium (28404, Stemcell Technologies) using the FACSaria III (BD Biosciences) and cultured at 37 °C with 5% CO₂, and colonies were counted on day 15 according to the manufacturer's instructions.

Cell lines. Molm13 and TF1 cells were purchased from the German Collection of Microorganisms and Cell Cultures and cultured at 37 °C with 5% CO₂ in RPMI 1640 medium (R8758, Sigma) supplemented with 10% FCS and 1% penicillin and streptomycin. Knockout of BIRC2 and BIRC4 (XIAP) by Cas9 ribonucleoprotein complexes. Preparation of Cas9 protein-gRNA ribonucleoprotein complexes (Cas9 RNPs) and electroporation of Cas9 RNPs was performed as previously described⁶⁸ with some modifications. Chemically modified CRISPR RNA (AltR-crRNA, Integrated DNA Technologies) targeting the BIRC2 and BIRC4 (XIAP) genes (5'-ATGTTTTGATA CGAGGGACC-3' and 5'-CATCAACACTGGCAGGAGCA-3', respectively) and tracrRNA (AltR-tracrRNA, Integrated DNA Technologies) were annealed in nuclease-free duplex buffer (11-01-03-01, Integrated DNA Technologies) as recommended by the supplier and stored at -20 °C until use. To generate Cas9 RNPs, 2 µg Cas9 protein (TrueCut Cas9 protein version 2, A36498, Thermo Fisher Scientific) was incubated with BIRC2 and BIRC4 (XIAP) crRNA-tracrRNA, at 20 nM each, in the duplex buffer at room temperature for 15 min. Immediately, Cas9 RNP was transduced into Molm13 and TF1a cells using the Neon electroporation system (1,350 V, 50-ms pulse width, three pulses per sample). Knockout efficiencies were analyzed by sequencing the genomic region targeted by the BIRC2 and BIRC4 (XIAP) gRNA species⁶⁹ and showed knockout rates of 62–63% and 48–49% for BIRC2 and BIRC4 in Molm13 and TF1 cells, respectively.

Efficiency of genome editing. Tracking of Indels by DEcomposition⁶⁹ was performed to evaluate efficiency of genome editing after electroporation of Cas9 RNPs. Genomic DNA was extracted using the Wizard Genomic DNA Purification kit (Promega) from cells transfected with non-targeting gRNA and those transfected with both BIRC2 and BIRC4 (XIAP) gRNA. To amplify the genomic region carrying the gRNA-targeting site, the following primers were used: BIRC2 forward (5'-AGTGCATCCACCTTTCAGGG-3'), BIRC2 reverse (5'-TCAGGCCACAACAGAAGCAT-3'); BIRC4 forward (5'-GCCTGCTTAAATATTACTTTCCTCA-3'), BIRC4 (XIAP) reverse (5'-TTTGTAGACTGCGTGGCACT-3'). PCR was conducted using GoTaq Hot Start Polymerase (M5001, Promega) according to the manufacturer's instructions. The PCR conditions were 1 min at 95 °C (1×), followed by 45 s at 95 °C, 15 s at 55 °C and 1 min at 72 °C (32 cycles). The PCR products were purified using the QIAquick Gel Extraction kit (28704, Qiagen). Purified

PCR samples (20 ng) were analyzed by capillary sequencing, and genome deletions were identified using TIDE software (<http://shinyapps.datacurators.nl/tide/>). Panel kinase inhibition assay. Inhibition of a panel of 115 human kinases by AZD5582 was assayed by Carina Biosciences. The assay buffer contained 20 mM HEPES, 0.01% Triton X-100, 1 mM dithiothreitol (pH 7.5) and 5 mM MgCl₂ (an additional 1 mM Mn for CSK and EGFR). The assay mixture contained 30 or 100 nM AZD5582 with 1 mM substrate and ATP (at K_m of 5–100 mM for each kinase). The kinase reactions were performed at room temperature for 1 h (5 h for CSK) and stopped by the addition of termination buffer (QuickScout Screening Assist MSA, Carina Biosciences). The phosphorylated and unphosphorylated peptides obtained were separated and quantified with the LabChip 3000 system (PerkinElmer). Background signal was observed in the no-enzyme negative control sample, which was set as 100% inhibition. Signal obtained from positive control samples containing enzyme, without AZD5582 treatment, was set as 0% inhibition. Inhibition of kinases by AZD5582 was calculated using signals observed in the test sample containing enzyme and AZD5582, measured in duplicate.

Serum chemistries. Pre- and post-treatment serum were obtained from mice treated with the combination of AZD5582 and venetoclax for 4 weeks, and serum chemistry analysis was performed using L-type CRE-M (for creatinine), L-type AST-J2 (for aspartate transaminase), L-type ALT-J2 (for alanine transaminase), NATURE CANCER L-type Amylase (for amylase) and L-type CHO-M (for total cholesterol) reaction kits (all from Wako), UN-S (for blood urea nitrogen, Denka Seiken), Nescooat VL T-BIL (for total bilirubin, Alfresa Pharma) and Quickauto-Neo GLU-HK (for glucose, Shino-Test).

Immunohistochemistry. Tissue sections (3 μ m) were cut from 4% paraformaldehyde- fixed paraffin-embedded recipient organs. Sections were deparaffinized using xylene and ethanol, and antigen retrieval was performed (Retrievagen A (pH 6.0), BD Pharmingen). Non-specific background was reduced by incubating the slides in methanol and H₂O₂ (Wako). After blocking with horse serum, slides were incubated with mouse anti-hCD45 antibody (Dako, M0701) (1:150) and then HRP-conjugated horse anti-rabbit or anti-mouse IgG antibody (ImmPRESS, MP-7500). Slides were then stained with 3,3'-diaminobenzidine or hematoxylin and eosin, dehydrated, mounted using VectaMount (Vector Laboratories) and analyzed with the Axiovert 200 microscope (Zeiss). Photos were taken using the AxioCam MRc 5 (Zeiss) and AxioVision release 4.6 software.

Statistical analysis. Gene expression levels between two experimental groups in vitro were tested by unpaired or paired two-tailed Student's t-tests and Wilcoxon signed-rank tests as described in the figure legends. The impact of DNA mutations and chromosomal aberrations were evaluated by two-tailed P values by Fisher's exact test. For in vivo treatment experiments, differences in the percentages of hCD45+ cells in PB, BM and spleen between the treatment groups were analyzed using paired two-tailed Student's t-tests, Wilcoxon signed-rank tests, unpaired two-tailed Student's t-tests or Welch's two-tailed t-tests as described in the figure legends. P values less than 0.05 were considered significant. Data are given as mean \pm s.e.m. or as median \pm interquartile range as described in the figure legends. Student's t-tests, Welch's two-tailed t-tests, Wilcoxon signed-rank tests and Fisher's exact tests were performed in GraphPad Prism version 8.3.1 (GraphPad Software) or R version 3.5.3. DEG analysis and motif analysis were conducted using R version 3.5.3 and Python version 3.6.1, respectively.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article. Data availability All DNA and RNA sequencing datasets produced in this study were deposited at the National Bioscience Database Center. Accession numbers are hum0116 for DNA sequencing data and hum0243 for RNA-seq data. Differential expression

analysis results can be browsed interactively on ZENBU at https://fantom.gsc.riken.jp/zenbu/reports/#Identification_of_therapeutic_targets_in_poor_outcome_AML_patients. Any other relevant data are available from the corresponding author upon reasonable request. Source data are provided with this paper. Code availability The scripts for motif activity analysis are available at http://fantom.gsc.riken.jp/5/suppl/Alam_et_al_2020/34. Received: 17 April 2020; Accepted: 22 January 2021; Published online: 18 March 2021

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