HMGA2 expression defines a subset of human AML with immature transcriptional signature and vulnerability to G2/M inhibition

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High-mobility group AT-hook 2 (HMGA2) is a nonhistone chromatin-binding protein that is normally expressed in stem cells of various tissues and aberrantly detected in several tumor types. We recently observed that one-fourth of human acute myeloid leukemia (AML) specimens express HMGA2, which associates with a very poor prognosis. We present results indicating that HMGA2+ AMLs share a distinct transcriptional signature representing an immature phenotype. Using single-cell analyses, we showed that HMGA2 is expressed in CD34+ subsets of stem cells and early progenitors, whether normal or derived from AML specimens. Of interest, we found that one of the strongest gene expression signatures associated with HMGA2 in AML is the upregulation of G2/M checkpoint genes. Whole-genome CRISPR/Cas9 screening in HMGA2 overexpressing cells further revealed a synthetic lethal interaction with several G2/M checkpoint genes. Accordingly, small molecules that target G2/M proteins were preferentially active in vitro and in vivo on HMGA2+ AML specimens. Together, our findings suggest that HMGA2 is a key functional determinant in AML and is associated with stem cell features, G2/M status, and related drug sensitivity.

Introduction

High-mobility group AT-hook 2 (HMGA2) is a nonhistone chromatin-binding protein known as an architectural transcriptional factor. Without any transcriptional activity of its own, HMGA2 binds to the minor groove of AT-rich DNA sequences to alter the chromatin structure and acts, positively or negatively, on the transcription of target genes.1 HMGA2 regulates numerous pathways in a context-dependent manner, which include cell cycle control, DNA repair, E2F, NF-KB, transforming growth factor-β, and the epithelial–mesenchymal transition.2 Although its expression is silenced in most human adult tissues,3,4 HMGA2 is ubiquitously expressed during embryogenesis, where it is critical for development and cell growth. Abnormal re-expression of HMGA2 in several solid neoplasms has been reported and is linked to chemo-resistance, advanced tumor grade, and poor prognosis.5-8

Key Points
- HMGA2 expression associates with immature cells in normal and leukemic context.
- Poor prognosis HMGA2+ AMLs share a unique transcriptional signature and sensitivity to G2/M inhibitors.
In mouse and human hematopoiesis, HMGA2 is preferentially expressed in hematopoietic stem and progenitor cells (HSPCs), at fetal and adult stages.10 Previous reports showed that HMGA2 promotes expansion of myeloid progenitors11 and that its overexpression in mouse bone marrow (BM) leads to a growth advantage and clonal expansion of HSPCs.12 Overexpression of HMGA2 in transgenic mice is also sufficient to induce tumor development, including hematologic malignancies.13-15

We previously showed that elevated HMGA2 levels identify a subgroup of AML patients with lower frequency of complete remission, higher frequency of relapse, and resistance to induction therapy.16 We established that HMGA2 expression predicts poor clinical outcome in AML, independently of cytogenetic risk, and that it represents a novel prognosis marker for this disease. Indeed, positivity for HMGA2 in high-risk AML patients further decreases the prognosis to near zero, defining a very-high-risk category for which no curative therapy is available.

In this report, we studied HMGA2 expression at the single-cell level and show that HMGA2 is predominantly expressed in stem and early progenitors, whether normal or leukemic. Using CRISPR/Cas9 whole-genome screening, we identified vulnerabilities of HMGA2 overexpressing leukemic cells in a cell cycle G2/M checkpoint. This finding was further confirmed in vitro and in vivo with the use of chemical inhibitors and may provide new therapeutic avenues for this poorly curable disease.

Methods

Study approval

The Leucegene project is an initiative approved by the Research Ethics Boards of Université de Montréal and Maisonneuve-Rosemont Hospital. All leukemia samples and paired normal DNA specimens were collected and characterized by the Quebec Leukemia Cell Bank after obtaining an institutional Research Ethics Board–approved protocol with informed consent according to the Declaration of Helsinki. The Quebec Leukemia Cell Bank is a biobank certified by the Canadian Tissue Repository Network.

Cytogenetic analyses and cohort definitions

Cytogenetic aberrations and composite karyotypes of the Leucegene cohort were described according to the International System for Human Cytogenomic Nomenclature 2016 guidelines.17 Complex karyotype was defined as having ≥3 clonal chromosomal abnormalities in the absence of World Health Organization–designated recurrent genetic abnormalities, including t(8;21), inv(16) or t(16;16), t(9;11), t(6;9), inv(3) or t(3;3), and AML with BCR-ABL1.18

Whole-genome CRISPR/Cas9 screen

The extended-knockout pooled lentiviral library composed of 278,754 single-guide RNAs (sgRNAs) targeting 19,084 RefSeq genes, 3872 hypothetical open reading frames, and 20,852 alternatively spliced isoforms developed by Bertomeu et al19 was used for whole-genome CRISPR/Cas9 screen. This library, for which each gene is targeted by ~10 sgRNAs, was introduced within a clone of the OCI-AML5 cell line expressing a doxycycline-inducible Cas9. Cells were infected at a multiplicity of infection of 5 with HMGA2-YFP and control YFP lentiviral vectors (backbone, MNDU-pgk-YFP) in media supplemented with polybrene for 48 hours. Infection efficiency, determined by the percentage of YFP cells, was monitored by using flow cytometry. The extended-knockout library (kept at a minimum of 500 cells per sgRNA) was then cultured in 10% fetal bovine serum Dulbecco’s modified Eagle medium supplemented with 2 μg/mL doxycycline for a period of 7 days to induce knockouts. The infected library was maintained in culture 7 more days without doxycycline. Cell concentration and percentage of YFP cells were assessed every 2 days. Finally, genomic DNA was extracted by cell lysis in buffer containing 50 mM Tris, 50 mM EDTA, and 1% sodium dodecyl sulfate and treated with proteinase K followed by RNase and then precipitation of proteins with 7.5 M ammonium acetate and isopropanol precipitation of genomic DNA. sgRNA sequences were recovered and fitted with Illumina adaptors by polymerase chain reaction and next-generation sequencing performed on an Illumina HiSeq 2000 device as previously described.19 Resulting reads were trimmed by using Trim Galore (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and aligned to the sgRNA sequences by using Bowtie aligner version 2.3.3.20 Synthetic rescue/positive selection and synthetic lethality/negative selection β scores, as well as statistical significance, were determined by using the MAGeCK-VISPRMAGeCK-MLE method.21

Primary AML sample culture and chemical screens

Freshly thawed primary AML specimens were used for chemical screens. Cryopreserved cells were thawed at 37°C in Iscove modified Dulbecco medium containing 20% fetal bovine serum and DNase I (100 μg/mL). Cells were resuspended in Iscove modified Dulbecco medium supplemented with 15% BIT (bovine serum albumin, insulin, and transferrin; Stemcell Technologies), 100 ng/mL...
Figure 2.
performed. Control wells received DMSO (0.1%) only. Cell viability
of inhibition for dose-response curves was calculated as 100
seeded cells in serial dilutions (8 dilutions, 1:3, 10
fl)
also observed in proliferative megakaryocyte progenitor cells (CD34−
engraftment was assessed by using the following antibodies: anti-
Statistical analyses
Results
HMGA2 is expressed in hematopoietic stem cells, early progenitors, and erythroid lineage
In vivo treatments on patient-derived xenograft models

Figure 2(continued) HMGA22lo AML share an immature transcriptional signature. (A) Dot plot representation of HMGA2 and CD34 expression assessed by total RNA-sequencing in the Leucegene cohort of 452 primary AML specimens. Log-transformed scale is used to better visualize AMLs with low expression of these markers.
Figure 3. Whole-genome CRISPR/Cas9 screen identifies cell cycle regulation as a vulnerability in HMGA2-expressing cells. (A) Western blot validation of HMGA2 protein expression in Cas9 OCI-AML5 15 days after infection with HMGA2-YFP and YFP vectors. (B) After infection with HMGA2-YFP– and YFP-expressing vectors, the percentage of YFP-expressing cells was monitored by flow cytometry during the course of CRISPR/Cas9 whole-genome screen. (C) Schematic overview of the CRISPR/Cas9 whole-genome screen performed in Cas9 OCI-AML5 cells containing the Extended Knockout sgRNA library. (D) Volcano plot representing results of whole-genome CRISPR/Cas9 screen performed in OCI-AML5 cells overexpressing HMGA2 compared with YFP control vector. Most significant genes belonging to the Gene Ontology (GO) terms 1901987 (regulation of cell cycle phase transition, P value \(5.0 \times 10^{-16}\), false discovery rate [FDR] q-value \(1.1 \times 10^{-13}\) and 0006281 (DNA repair, P value \(1.2 \times 10^{-13}\), FDR q-value \(9.9 \times 10^{-11}\) that enriched in synthetic lethal interaction are depicted. ATR, CHEK1, and WEE1 are also depicted. Asterisks indicate genes belonging to both GO terms. (E) Exhaustive list of significant GO terms associated with synthetic lethality in cells overexpressing HMGA2 compared with YFP control cells. (F) Gene Set Enrichment Analysis plot comparing HMGA2 high (RPKM >2; n = 39) vs HMGA2 null (RPKM = 0; n = 83) transcriptomic signatures in primary AML specimens. Results obtained for G2/M checkpoint hallmark gene set. DOX, doxycycline; ER, endoplasmic reticulum; NES, normalized enrichment score.
Figure 4.
becomes more prominent in proliferative CD34+ FLT3+ progenitors in culture, concomitant with MKI67 expression (orange vs red circles in Figure 1E and supplemental Figure 1D-E). Again, CD34+ AVP+ HMGA2+ HSC candidates remain mostly negative for MKI67 in cultured cells.

Matching a recent report by Kumar et al.,10 we observed that HMGA2 silencing strongly impaired human CD34+ cell engraftment in mice (Figure 1F). This finding suggests that HMGA2 is not only expressed but also essential to sustain human HSCs in vivo.

**HMGA2** **high** AML share an immature transcriptional signature

To assess the functional role of HMGA2 in AML, we looked at HMGA2 expression in the Leucegene cohort comprising 452 primary AML specimens representative of the AML subgroup diversity. HMGA2 expression comprised between 0 and 17.1 reads per kilobase of transcript per million reads mapped (RPKM) (log transformed scale, Figure 2A), and we thus defined extreme groups of AML samples with HMGA2 null (RPKM = 0; n = 83 or 18.4% of the cohort) or HMGA2 high (RPKM ≥2; n = 39 or 8.6% of the cohort) expression. As found with normal CD34+ cells, Gene Set Enrichment Analysis revealed a strong enrichment for an HSC gene signature in HMGA2high AMLs (Figure 2B; supplemental Table 1), again indicating a stem cell–like identity associated with this gene. Multidimensional scaling (MDS) analysis also showed that specimens with high HMGA2 expression cluster together, independently of their genetic subgroup affiliation, suggesting that a specific HMGA2 transcriptional signature exists in AML and that HMGA2high AML represents a distinct entity (Figure 2C-D).

We next investigated whether HMGA2 expression in HMGA2high AMLs was caused by cancer-related anomalies such as gene translocation, gene amplification, or 3’ untranslated region (3’ UTR) truncation.5,25-28 To address this, we analyzed the HMGA2 locus in HMGA2high AMLs by low-pass whole-genome sequencing and transcriptomics, and did not detect any gene amplifications (supplemental Figure 2A), fusions, or 3’ UTR truncations that could explain the aberrant HMGA2 expression observed in a subset of our AML cohort.

Interestingly, single-cell RNA-sequencing analysis of primary AMLs (from Petti et al.29) showed that, when expressed, HMGA2 is restricted to a subset of CD34– leukemic cells (Figure 2E; supplemental Figure 2B). As observed in normal hematopoiesis, HMGA2 expression in leukemic cells was observed in both noncycling cells—that coexpress the primitive marker AVP, lack PCNA expression as well as differentiation markers such as ELANE and FCGR3A (likely quiescent stem cells [black arrow in Figure 2E and supplemental Figure 2C])—and in cycling progenitor cells (red arrow in Figure 2E and supplemental Figure 2C). Together, these data suggest that HMGA2 expression in AML is determined by the cell of origin rather than acquired through genetic events and points to a particular leukemia subset that originates from primitive CD34+ BM cells. To support this, HMGA2high AMLs display a HMGA2 expression level comparable to that of primitive CD34+ and CD34+CD45RA– human cells (supplemental Figure 2D).

**Genome-wide search for vulnerabilities associated with HMGA2 expression**

HMGA2+ AMLs remain extremely difficult to cure,16 representing a true unmet medical need. To identify vulnerabilities linked to this AML subgroup, we turned to genome-wide approaches. First, we looked at genetic dependencies and small molecule sensitivities associated with HMGA2 using the Cancer Dependency Map (DepMap). However, this showed poor codependencies, with the best gene (MSRB3) presenting a Pearson correlation of only 0.28 with HMGA2. Next, we engineered an AML cell line overexpressing HMGA2 (OCI-AML5) (Figure 3A), whose proliferation was not altered by this manipulation (Figure 3B), and conducted a whole-genome CRISPR/Cas9 screen in these cells with a particular focus on synthetic lethal genes (Figure 3C). Focusing on genes conferring a growth disadvantage, several pathways with potential for therapeutic intervention were identified (Figure 3D-E; supplemental Table 2). In line with the known functions of HMGA2 in the stability of replication forks30 and telomeres,31 we found synthetic lethal interactions between HMGA2 overexpression and the loss of genes involved in DNA replication and telomere maintenance. Interestingly, this genomic screen also identified synthetic lethal interactions between HMGA2 and the loss of genes involved in cell cycle regulation and DNA repair. In particular, disruption of genes regulating cell cycle phase transition appeared critical, which is further supported by a role of HMGA2 in maintaining genome stability.32,33 In line with the upregulation of a G2/M checkpoint gene signature identified in HMGA2high primary AML specimens (Figure 3F), G2/M cell cycle phase transition was also pinpointed in this CRISPR screen.

In contrast, fewer genes were identified that conferred a growth advantage (ie, synthetic rescue) when disrupted concomitantly with HMGA2 overexpression (supplemental Table 2). This included a known HMGA2-interacting partner, PARK2 (Parkin E3 ubiquitin-protein ligase)34; however, no significant enrichment for canonical pathways was observed.

**G2/M checkpoint inhibitors are particularly active on HMGA2+ AMLs**

To further investigate results obtained from the CRISPR/Cas9 screen, we screened a selected library of cell cycle and DNA damage protein inhibitors (Figure 4A) on 38 primary AML specimens (Figure 4B). Tested samples included complex karyotype (CK) and intermediate-risk AML specimens chosen from the Leucegene cohort according to their TP53 and HMGA2 status. Overall results

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![Figure 4](https://example.com/figure4.png) **Figure 4 (continued) Increased sensitivity of HMGA2+ primary AMLs toward G2/M checkpoint inhibitors.** (A) List of inhibitors and their primary target(s) used in the chemical screen. Genotoxic agents used as controls are not listed. (B) Repartition of the primary AML specimens selected and screen layout. Volcano plot showing the differential compound sensitivity in CK vs intermediate-risk AML specimens (C), TP53 wild-type (WT) vs TP53 altered AML specimens (D), and CK HMGA2+ vs CK HMGA2+ samples (E). White dots represent genotoxic agents. (F) Dot plot comparison of representative ATR, CHK1, and WEE1 inhibitors between intermediate, CK HMGA2+, and CK HMGA2+ specimens (median is depicted, Mann-Whitney U test). (G) CHEK1, WEE1, ATM, and CHEK2 messenger RNA expression in HMGA2 null (RPKM = 0; n = 83) and HMGA2 high (RPKM >2; n = 39) AML samples (median is depicted, Mann-Whitney U test). 5-FU, 5-fluorouracil; ns, not significant; PARP, poly(ADP-ribose) polymerase.
Figure 5.
showed strong antiproliferative activity of CHK1 and dual CHK1/CHK2 inhibitors on primary specimens, contrasting sharply with the lack of activity of selective CHK2 inhibitors (supplementary Figure 3A-B; supplemental Table 3). The most active compounds were also directed against ATR (VE-822), WEE1 (MK-1775), poly(ADP-ribose) polymerase (talazoparib), and phosphatidylinositol 3-kinase (P13K; P1-103) activity, whereas ATM, DNA-dependent protein kinase (DNA-PK), CHK2, replication protein A (RPA), and broad DNA-PK/P13K inhibitors did not show substantial activity on these primary specimens.

Comparison of sensitivity profiles of CK and intermediate-risk AML revealed no differential sensitivity to potent DNA damage inhibitors (Figure 4C). Not surprisingly, we found that TP53-altered specimens were more resistant to DNA-damaging agents, including drugs used in anticancer therapy (Figure 4D), and none of the DNA damage protein inhibitors were selectively active toward TP53-altered AML specimens. Interestingly, however, we observed that HMGA2 CK specimens were more sensitive to ATR, CHK1, and WEE1 inhibitors. Conversely, compounds active on CHK2, DNA-PK, poly(ADP-ribose) polymerase, RPA, and P13K showed no specificity toward HMGA2 CK specimens (Figure 4E-F). It is worth noting that ATR and CHK1 kinases belong to the same DNA damage-signaling pathway, which is critical for DNA repair and G2/M checkpoint transition, and WEE1 acts downstream by regulating mitotic entry and integrating signals from the ATR/CHK1 checkpoint (supplementary Figure 3C). HMGA2 CK high AML specimens expressed elevated levels of CHEK1 and WEE1, whereas ATM and CHEK2 expression levels were not affected by HMGA2 status (Figure 4G). Expression of the adaptors Claspin and TOPBP1 that facilitate CHK1 phosphorylation by ATR were also increased in HMGA2 CK high samples without affecting ATM levels (supplemental Figure 3D). Together with the CRISPR/Cas9 screen results, our data suggest that the G2/M cell cycle transition, targeted genetically or pharmacologically, is a potential therapeutic target in HMGA2 CK AML.

**HMGA2 overexpression in cell lines modulates sensitivity to G2/M checkpoint inhibitors**

Using lentiviral gene transfer, HMGA2 was overexpressed in several hematopoietic cell lines, including K562, OCI-AML5, and THP-1, to investigate whether manipulating HMGA2 levels can modulate sensitivity to G2/M checkpoint inhibitors. Of note, the impact of HMGA2 expression on proliferation and cell cycle profile was absent or very limited (Figure 5A-C; supplemental Figure 4A-C, 4E-G). We found that cell lines engineered to overexpress HMGA2 had a twofold to sevenfold increase in sensitivity to ATR and CHK1 inhibitors (Figure 5D-E; supplemental Figure 4D-H; supplemental Table 4). Importantly, results appeared p53 independent, as similar patterns were obtained using TP53 wild-type (OCI-AML5) and TP53-deficient (K562 and THP-1) cells. Overall, these results suggest that HMGA2 levels modulate sensitivity to G2/M checkpoint inhibitors.

In addition, we observed that PLK1 expression is significantly higher (P = .018) in HMGA2 CK high AML samples than in those that do not express this gene (Figure 5F). HMGA2 overexpression in 2 different cell lines also confers enhanced sensitivity to 2 potent polo-like kinase 1 (PLK1) inhibitors (threedfold and sevenfold for volasertib and fourfold and fivefold for GSK46136) (Figure 5G-H). These data are interesting in the context that PLK1 is a key player in G2 phase and mitosis initiation and that CK AMLs, which frequently express HMGA2, are also sensitive to PLK1 inhibition. Together, these results are consistent with the hypothesis that HMGA2 expression sensitizes cells to molecules that target G2/M transition.

**HMGA2 CK high PDXs are sensitive to PLK1 inhibitors in vivo**

To gain insights on the in vivo response of HMGA2 CK high AML to PLK1 inhibition, we transplanted 2 PDX specimens in NSG mice and monitored response to AraC and to volasertib, a clinically available and potent PLK1 inhibitor. PDX 05H179 and 09H057 were derived from an EVI1 rearranged and a CK specimen, respectively, and both express high levels of HMGA2 (RPKM = 11.8 and 6). After confirmation of engraftment by PDX cells, animals were treated with AraC, volasertib, or control vehicle. For PDX 05H179 (Figure 6A-B), development of leukemia in mice was monitored by measurement of human CD45 cells in BM at days 10 and 28 after the onset of treatment. Although leukemia engraftment progressed in vehicle- and AraC-treated mice, volasertib was able to contain leukemia development. For PDX 09H057 (Figure 6C-D), treatments were initiated early after transplantation and animals followed up for a long period of time to monitor the effectiveness of treatments at early stages and development of leukemia from therapy-resistant cells. At day 78 posttreatment initiation, this leukemia clearly progressed in the majority of the mice analyzed 10 weeks after the mice treated with volasertib. These data indicate that the percentage of leukemia engraftment remained high AML samples than in those that do not express this gene (Figure 5F). HMGA2 overexpression in 2 different cell lines also confers enhanced sensitivity to 2 potent polo-like kinase 1 (PLK1) inhibitors (threedfold and sevenfold for volasertib and fourfold and fivefold for GSK46136) (Figure 5G-H). These data are interesting in the context that PLK1 is a key player in G2 phase and mitosis initiation and that CK AMLs, which frequently express HMGA2, are also sensitive to PLK1 inhibition. Together, these results are consistent with the hypothesis that HMGA2 expression sensitizes cells to molecules that target G2/M transition.

**Discussion**

In solid tumors, gene translocation, gene amplification, or 3' UTR HMGA2 truncations have been reported to express aberrant HMGA2 expression. In hematologic malignancies, however, only a few
Figure 6. Poor prognosis HMGA2<sup>high</sup> PDXs are sensitive to PLK1 inhibitors in vivo. Summary of the treatment protocols and time point analysis for NSG mice transplanted with 2 million of 05H179 (A) or 09H057 (C) AML cells. Two weeks after transplantation, mice were treated with AraC (50 mg/kg, 5 days per week during 1 week), volasertib (10 mg/kg, 2 days per week during 4 weeks), or vehicle. BM aspiration was performed at indicated times. (B) Percentage of human CD45<sup>+</sup> cells in BM at day 10 and 28 after AraC, volasertib, or vehicle treatment (n = 5, median is depicted; t test). (D) Percentage of human CD45<sup>+</sup> (huCD45<sup>+</sup>) cells in BM at days 28, 78, and 94 after AraC, volasertib, or vehicle treatment (n = 5, median is depicted; t test). ns, not significant.
cases of chromosomal rearrangements at the HMGA2 locus have been reported, and none detected in our AML cohort. Rather, our data suggest that HMGA2 expression reflects a stem cell origin or a more immature state of these leukemias. Looking at single-cell analyses, we confirmed that HMGA2 expression in normal hematopoiesis is predominant in HSPCs, early progenitors, and the erythroid lineage. In AML, a similar pattern was observed with a marked expression of HMGA2 in the most immature cells while absent in differentiated cell clusters. As a result of this restricted expression, HMGA2 protein could not be detected at the protein level in bulk primary AML specimens.

Our group reported that HMGA2 is expressed in most CK AMLs; nevertheless, elevated HMGA2 expression can occur in a non-CK background. In addition, MDS-based gene expression clustering showed that HMGA2-high specimens cluster together, independent of their karyotype or genetic group, and that HMGA2 is a prognostic marker independent of the cytogenetic risk. Altogether, these data suggest that the transcriptional regulator HMGA2 could have an important imprint in AML and define a distinct AML identity.

HMGA2 has become an attractive therapeutic target, but due to its nature, HMGA2 is not considered an easily druggable protein. Minor groove-binding agents such as netropsin and trabectedin displace HMGA2 from its target promoters and achieve antitumoral activity but lack specificity. Targeting microRNAs or downstream pathways activated by HMGA2 represent alternative approaches to be explored. Tan et al. indeed reported that HMGA2 activates the PI3K/protein kinase B/mammalian target of rapamycin pathway in HL60 and NB4 cells and promotes AML proliferation. By conducting a whole-genome CRISPR/Cas9 screen in leukemic cells, we identified cell cycle regulation and DNA repair as synthetic lethal interactions with HMGA2 overexpression. These results highlighted a vulnerability of HMGA2 overexpressing cells toward cell cycle regulation. Accordingly, we found that the strongest gene expression signature associated with HMGA2 in AML is the upregulation of G2/M checkpoint genes. It has also been reported that HMGA2 interacts with the base excision repair machinery and protects cancer cells from DNA lesions induced by chemotherapeutic agents, and that HMGA2 expression increases ATR/CHK1 phosphorylation in cancer cell lines and helps maintain genome stability upon DNA damage. Interrogation of a DNA damage inhibitor library on primary AML specimens indeed confirmed an increased sensitivity of HMGA2+ AML to ATR, CHK1, and WEE1 inhibitors. These data suggest that HMGA2 expression, irrespective of the TP53 status, is of importance regarding the response to G2/M inhibitors in AML.

Together, our results showed that HMGA2 expression in AMLs associates with the most immature leukemic cells, a distinct transcriptional profile, and sensitivity to agents targeting G2/M transition. Because some of these agents have been or are currently under evaluation in clinical trials, our results suggest that these trials may benefit from targeted subgroup analyses and in particular patients with HMGA2+ AML. The recently developed and reliable clinical test for this gene would be useful in this context.

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Authorship

Contribution: C.M. contributed to project conception and single-cell data analysis, performed and analyzed CRISPR/Cas9 and chemical screens, generated corresponding material, and wrote the manuscript; J.-F.S. contributed to project conception, MDS, and CRISPR/Cas9 screen analyses; J.C. contributed to project conception, single-cell data analysis, and CRISPR/Cas9 screen; V.-P.L. contributed to genomic and chemical screen analysis; B.L. contributed to single-cell data analysis; C.T. performed primary AML chemical screen; I.B. contributed to chemical screen and data validation; N.M. contributed to in vivo studies; T.M. contributed to CRISPR/Cas9 screen; A.M. is responsible for the chemistry team as part of the Leucegene project; J.H. selected and provided all CRISPR/Cas9 analyses; J.C. contributed to project conception and supervision, and cowrote the paper.

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