RAS mutations drive proliferative chronic myelomonocytic leukemia via a KMT2A-PLK1 axis

Ryan M. Carr1,2,13, Denis Vorobyev3,13, Terra Lasho1, David L. Marks2, Ezequiel J. Tolosa2, Alexis Vedder4, Luciana L. Almada2, Andrey Yurchenko3, Ismael Padoleau3, Bonnie Alver5, Giacomo Coltro1, Moritz Binder1, Stephanie L. Safgren2, Isaac Horn2, Xiaona You6, Eric Solary7, Maria E. Balasis4, Kurt Berger8, James Hiebert1, Thomas Witzig1, Ajinkya Buradkar1, Temeida Graf9, Peter Valent9,10, Abhishek A. Mangaonkar1, Keith D. Robertson5, Matthew T. Howard11, Scott H. Kaufmann1, Christopher Pin8, Martin E. Fernandez-Zapico2, Klaus Geissler12, Nathalie Droin7, Eric Padron4, Jing Zhang6, Sergey Nikolaev3✉ & Mrinal M. Patnaik1✉

Proliferative chronic myelomonocytic leukemia (pCMML), an aggressive CMML subtype, is associated with dismal outcomes. RAS pathway mutations, mainly NRASG12D, define the pCMML phenotype as demonstrated by our exome sequencing, progenitor colony assays and a Vav-Cre-NrasG12D mouse model. Further, these mutations promote CMML transformation to acute myeloid leukemia. Using a multiomics platform and biochemical and molecular studies we show that in pCMML RAS pathway mutations are associated with a unique gene expression profile enriched in mitotic kinases such as polo-like kinase 1 (PLK1). PLK1 transcript levels are shown to be regulated by an unmutated lysine methyl-transferase (KMT2A) resulting in increased promoter monomethylation of lysine 4 of histone 3. Pharmacologic inhibition of PLK1 in RAS mutant patient-derived xenografts, demonstrates the utility of personalized biomarker-driven therapeutics in pCMML.
Chronic myelomonocytic leukemia (CMML) is an aggressive hematological malignancy characterized by sustained peripheral blood (PB) mononuclearosis (absolute monocyte count/AMC ≥ 1 x 10^9/L, with monocytes ≥10% of the white blood cell count differential), bone marrow (BM) dysplasia and an inherent risk for leukemic transformation (LT) to secondary acute myeloid leukemia (sAML). The only curative modality, allogeneic hematopoietic cell transplantation (HCT), is rarely feasible because of age (median age at diagnosis 73 years) and comorbidities2–4. Patients not eligible for HCT receive symptom-guided therapies, including hypomethylating agents (HMA)2,5. While HMA epigenetically restore hematopoiesis in a subset of CMML patients, they fail to significantly alter the disease course or impact mutational allele burdens5,6. Finally, relative to patients with de novo AML, survival in sAML is much shorter (<4 months), even with the use of AML-directed therapies. Thus, there is an urgent and unmet need for rationally developed therapies for patients with CMML.

The 2016 iteration of the World Health Organization (WHO) classification of myeloid malignancies has called for the recognition of proliferative CMML (pCMML) and dysplastic (dCMML) subtypes based on a diagnostic white blood cell count (WBC) of ≥13 x 10^9/L for the former. This classification has often been criticized as being somewhat arbitrary and biological differences remain to be explored. The molecular fingerprint for CMML combines recurrent mutations in approximately 40 genes, some of which are associated with poor outcomes (ASXL1, NRAS, RUNX1, and SETBP1), and are incorporated into CMML-specific prognostic scoring systems8–10. Prior studies have demonstrated a higher frequency of RAS mutations (NRAS/KRAS) in pCMML; hypothesized to be secondary events and associated with poor outcomes11. Unresolved questions in CMML include whether there are true biological differences between pCMML and dCMML, how genetic and epigenetic events contribute to CMML progression and LT, and whether these molecular events render CMML vulnerable to specific therapeutic interventions.

In the present study, by using a combination of DNA sequencing, RNA-, and chromatin immunoprecipitation and sequencing (ChIP-seq), we demonstrate that pCMML and dCMML are independent biological entities, with genetic, transcriptomic, and epigenetic differences; with pCMML being defined by the presence of clonal RAS pathway mutations. Using whole and targeted exome sequencing, in vitro patient-derived progenitor colony forming assays and a Vav-Cre-NrasG12D mouse model, we demonstrate conclusively that clonal NRAS mutations can drive the pCMML phenotype. In clonal RAS pathway mutant pCMML, RAS pathway mutations result in increased expression of the mitotic checkpoint kinase PLK1 through the lysine methyltransferase KMT2A (MLL1). KMT2A mediates expression of PLK1 via monomethylation of histone 3 (H3) lysine 4 (K4) at the PLK1 promoter. While monomethylation of H3K4 is usually placed by KMT2C/2D, in clonal RAS pathway mutant pCMML, this histone mark is specifically regulated by an unmutated KMT2A. Further, pharmacological inhibition of PLK1 decreases monocytoysis and hepatosplenomegaly and improves hematopoiesis in patient-derived, RAS-mutant pCMML xenografts. These results support a role for clonal RAS pathway mutations in pCMML biology, CMML progression to AML; demonstrating oncogenic function of an unmutated KMT2A, laying a framework for rationally defined personalized therapies in CMML.

Results

Clonal RAS pathway mutations correlate with proliferative CMML. Outcome differences were analyzed between WHO-defined dCMML and pCMML subtypes. Univariate analysis of a cohort of 1183 Mayo Clinic-GFM-Austrian CMML patients (Supplementary Table 1; dCMML, 607 and pCMML, 576), median age 72 years (range, 18–95 years), 66% male, with a median follow-up of 50 months (range, 41–54.5 months) validated an inferior overall-survival (OS) of pCMML patients relative to dCMML (23 vs 39.6 months; p < 0.0001; Fig. 1A). This stratification also predicted a shorter AML-free survival in patients with pCMML versus dCMML (Fig. 1B, 18 vs 32 months; p < 0.0001). Genetic differences were assessed between the subtypes in 973 molecularly annotated Mayo Clinic-GFM-Austrian CMML patients (477 with pCMML and 496 with dCMML) using a targeted targeted next-generation sequencing (NGS) assay designed to detect mutations in oncogenic RAS pathway genes; NRAS, KRAS, CBL, and PTPN11. If note, while NFI is an important component of the RAS pathway1, it is the least frequently mutated RAS pathway gene in CMML and was not included in the NGS panels (Supplementary Fig. 1A)12. A higher frequency of NRAS (30% versus 8%; p < 0.001) and oncogenic RAS pathway mutations (46% versus 25%, p < 0.001) were identified in pCMML versus dCMML (Fig. 1C and Supplementary Table 2). Additional differences between pCMML and dCMML included a higher frequency of ASXL1 (60% vs 42%; p < 0.0001), JAK2V617F (12% vs 5%; p = 0.0001) mutations and trisomy 8 (11% vs 4%; p = 0.02) in pCMML, while TET2 (43% vs 54%; p = 0.002) mutations were more common in CMML (Supplementary Table 2). Phenotypically, pCMML patients had higher white blood cell (WBC) counts, absolute monocyte counts (AMC), peripheral blood (PB) blasts, and circulating immature myeloid cells (IMC), relative to dCMML patients (Supplementary Fig. 1B).

Patients stratified by the presence or absence of NRAS mutations demonstrated an inferior OS for NRAS mutant CMML versus NRAS wild type (24 vs 33 months; p = 0.0055; Fig. 1D). This impact was also retained for cumulative oncogenic RAS pathway mutations (Supplementary Fig. 1C). pCMML with NRAS mutations was the most aggressive CMML subtype, with significantly shorter OS (22 months; p = 0.0007) relative to all other CMML subtypes combined (33 months) (Fig. 1E). Notably, OS was not significantly different when comparing each CMML subtype stratified by NRAS mutational status (Supplementary Fig. 1D). Similarly, NRAS mutant pCMML had the shortest AML-FS (16 months; p = 0.0001) versus other subtypes combined (AML-FS 29 months) and individually (Fig. 1F and Supplementary Fig. 1E). In addition, based on variant allele frequency (VAF) analysis, in 977 CMML patients that underwent NGS, there was a higher frequency of clonal NRAS, KRAS and CBL mutations in pCMML relative to dCMML (Fig. 1G and Supplementary Fig. 1F).

We then estimated the odds ratios of driver mutations including NRAS selected by a L1-regularized logistic regression model to assess their impact on the pCMML/dCMML phenotype, LT risk, OS, and AML-FS (Fig. 1H, I). Mutations negatively impacting OS and AML-FS included NRAS, ASXL1, RUNX1, DNM3TA, and EZH2 (Fig. 1H, I). We then applied a multivariate cox model for prediction of OS using mutations identified above and then combined the mutational data with clinical variables known to be prognostic in CMML (Supplementary Fig. 1G–J)8–10. Analysis was first carried out for individual RAS pathway mutations and then for oncogenic (combined) RAS pathway mutations. Importantly, mutant NRAS alone did not reach statistical significance as an independent factor impacting OS or AML-FS. The combined oncogenic RAS pathway category was statistically significant in a model that only included genetic factors (HR = 1.2, CI: 1.08–1.33), while there was a trend towards inferior outcomes when assessed in a model that included genetic and clinical parameters (HR = 1.11, CI: 0.99–1.24).
Pairing whole-exome sequencing (WES) performed on BM or PB mononuclear cells (MNC) collected at both chronic phase CMML and sAML stages in a cohort of 48 patients were analyzed (Supplementary Table 3). Median OS was 25 months after diagnosis and 6.4 months after LT. At CMML diagnosis, or first referral to study institution, mutations in driver genes (detected in every patient; mean, 3.6 per patient) and somatic copy number alterations (SCNA; Fig. 2A, B and Supplementary Fig. 2A) were comparable to previous reports. Some mutations, such as in TET2, SRSF2, and ASXL1, were predominantly clonal while others, including the oncogenic RAS pathway, such as NRAS, KRAS, and CBL, showed a bimodal distribution of VAF (Fig. 2C).

Compared to chronic phase CMML, additional driver mutations (median = 2) were detected in 44% of LT samples (Supplementary Fig. 2B–E). The most striking changes included an increase in mutations of the oncogenic RAS pathway (from 52 to 67%), and in SCNA (from 52 to 75%; Fig. 2A, B and Supplementary Fig. 2).

Tracing clonal evolution from CMML onset to LT, we categorized driver mutations (Table S4 for definition of driver mutations) into those detected in CMMML and at LT (primary drivers or 1d), only in LT (secondary drivers, 2d) and those in CMML and lost in LT, suggesting sub clonal secondary drivers (3d; Supplementary Fig. 2D). Secondary drivers (2d and 3d) were
Fig. 2 Alterations in driver mutation profiles delineate stages of CMML progression. Whole exome analysis of 48 paired CMML and secondary AML (sAML) samples. 
A Prevalence of driver mutations and somatic copy number alterations (SCNAS) in CMML and sAML. RAS pathway represents all mutations in MAPK pathway. 
B Frequency of driver mutations and SCNAS in CMML and sAML. 
C Violin plots depicting variant allele frequencies (VAFs) for the most frequent driver mutations in CMML and sAML. 
D Frequency of driver mutations and SCNAS in CMML and sAML and their categorization as 1d, 2d, or 3d. 
E Genetic profiling of CMML evolution in eighteen patients at two time points by whole exome sequencing. 
F Odds ratios of genetic factors selected by the L1-regularized logistic regressions that have the greatest impact on dysplastic/proliferative CMML categorization and leukemic transformation (LT) risk. The x-axis represents odds ratios of LT risk (high or low). The y-axis represents categorization of low or high white blood cell (WBC) count, or pCMML vs dCMML classification. The horizontal and vertical size of ellipses reflects corresponding p-values calculated by two-tailed Fisher’s exact test. Additionally, confidence intervals (α = 0.05) of odds ratio values are presented. The mean is the measure of center. 
G Stratification of parameters associated with aggressive disease phenotype by number of driver mutations. 
H Fish plots derived from patient-derived single colony assays of representative cases of pCMML (left) and dCMML (right). Scatter plot to the left of each fish plot represents the genotype of individual colonies as determined by Sanger sequencing of the indicated genes. RAS mutation status is depicted on the x-axis. The arrow indicates inferred evolutionary trajectory from which the fish plot was derived.
significantly different from primary drivers (1d), i.e. average number of mutations per category (1d: 3.3; 2d: 0.8; 3d: 0.4) and the spectrum of mutated genes were distinct. TET2, ASXL1, SRSF2 mutations as well as monosomy 7 were almost exclusively 1d. NRAS was the main gene whose mutation rate increased from chronic phase to LT (Fig. 2D). The fraction of NRAS mutations detected in LT was significantly higher than that detected in CMML at diagnosis ($p = 0.0002$; Fig. 2D and Supplementary Fig. 2C). In 27% of cases, driver mutations were lost between chronic phase and LT. Eliminated sub clones harbored fewer driver mutations than progressive sub clones (19 vs 38; binomial test, $p = 0.01$) (Supplementary Fig. 2E).

In the WES cohort, CMML patients without RAS pathway mutations had a better OS, in comparison to those with RAS pathway mutations ($p = 0.045$; Supplementary Fig. 2F). WES of serial samples collected along disease evolution from 18 CMML patients (data set previously published) showed time-dependent accumulation of driver mutations affecting RAS pathway genes, including NRAS, KRAS, and CBL (Fig. 2E). In addition, in the combined WES cohort, NRAS mutations were most significantly associated with aggressive disease features, such as leukocytosis (OR = 3.0, CI: 1.7–5.1) and LT risk (OR = 2.7, CI: 1.4–5.3; Fig. 2F), with the number of driver mutations correlating with disease severity in CMML (Fig. 2G).

Of note, targeted sequencing of patient-derived progenitor colony forming assays indicated that mutations can accumulate in multiple orders, e.g. mutations in ASXL1 or SRSF2 can appear after those in NRAS or KRAS, which was the case in pCMML (Fig. 2H). Altogether, these analyses in independent patient cohorts indicate that enrichment on oncogenic RAS pathway mutations is typically associated with the pCMML phenotype, aggressive disease features and with transformation to sAML.

**Clonal NRAS/KRAS activation drives a proliferative CMML phenotype.** To explore the impact of RAS pathway mutations in CMML, we used patient-derived cells to perform progenitor colony forming assays. Transduction of NRAS wild-type dCMML MNC with a NRAS-expressing adeno virus resulted in significant increases in colony formation relative to empty vector controls (Fig. 3A). Electroporation of primary CMML samples was highly efficient based on green fluorescent protein controls (Supplementary Fig. 3A). NRAS$^{G12D}$ transfection in NRAS wild-type CMML MNC increased ex vivo proliferation whereas knockdown of NRAS in NRAS$^{G12D}$ MNC demonstrated the opposite effect (Fig. 3B). Knockdown of NRAS was highly effective as confirmed by Western blot (Supplementary Fig. 3B). Similarly, KRAS$^{G12D}$ transfection in KRAS wild-type CMML MNC promoted cell growth and KRAS$^{G12D}$ knockdown in KRAS$^{G12D}$ mutant CMML MNC (Supplementary Fig. 3C, D) lowered proliferation, indicating that the growth effects extended to other RAS pathway mutations. Finally, knockdown of JAK2 in JAK2 mutant, NRAS wild-type pCMML MNC resulted in significant reduction in cell proliferation, confirming its role in the proliferative phenotype in a subset of patients (Supplementary Fig. 3E).

Since RAS pathway mutations can also be seen in dCMML, we first demonstrated a clear correlation between NRAS mutant pCMML with higher risk phenotypic features and an inferior AML-FS, in comparison to NRAS mutant dCMML (Supplementary Fig. 1B, E). We then demonstrated that knockdown of NRAS$^{G12D}$ in NRAS mutant dCMML MNC did not have an impact on ex vivo proliferation (Supplementary Fig. 3F). Next, to better define clonal versus subclonal RAS pathway mutation thresholds, we assessed mutant NRAS and mutant oncogenic RAS pathway VAF data and correlated RAS VAF as a continuous measure of subclonality in relationship to clinical parameters. A receiver operating characteristic curve (ROC) analysis demonstrated that a NRAS VAF $\geq 0.3$ or an oncogenic RAS VAF of $\geq 0.19$ best correlated with a pCMML phenotype, in comparison to binary RAS mutation status (Fig. 3C–F). Finally, we showed that the NRAS VAF threshold of $\geq 0.3$ correlated with lower platelet counts (Supplementary Fig. 3G), while the RAS VAF threshold of $\geq 0.19$ correlated with lower platelet counts and higher PB blasts (Supplementary Fig. 3H, I); with thrombocytopenia and PB blasts being markers of higher risk disease.

To determine the in vivo effect of NRAS$^{G12D}$ on hematopoiesis, a genetically engineered Vav-Cre-Nras$^{G12D}$ mouse model on a C57Bl/6 background was developed. This was developed over the existing Mxl1-Cre-Nras$^{G12D}$ model given the mice develop a myeloproliferative disorder, but eventually die of a spectrum of hematological malignancies. The Mxl1-Cre transgene is active in the liver and hematopoietic cells after induction. Due to activity in the liver, mice develop hepatic histiocytic infiltrates and other phenotypes not specific to CMML. With Cre recombination under control of the Vav promoter, Nras$^{G12D}$ is more specifically induced in the hematopoietic system and phenocopies pCMML. At 6 weeks, these mice demonstrated a significant increase in PB and BM monocyte counts, without significant changes in absolute neutrophil counts (Fig. 3G). Analysis of ERK phosphorylation in BM progenitor cells (Lin$^{-}$c-kit$^{-}$) suggested increased sensitivity to GM-CSF relative to controls, previously described in human CMML (Fig. 3H). Histopathological analyses showed BM hypercellularity with megakaryocytic atypia/dysplasia and splenic red pulp hyperplasia, architectural distortion, and splenic extramedullary hematopoiesis (Fig. 3I and Supplementary Fig. 3J). BM MNC obtained from Vav-Cre-Nras$^{G12D}$ animals formed increased colony numbers versus control mice (Fig. 3J). Finally, Vav-Cre-Nras$^{G12D}$ mice had significantly shortened survival relative to control mice (median 400 days vs not reached; Fig. 3K). These data demonstrate that Nras$^{G12D}$ alone expressed in Vav + hematopoietic cells is sufficient to initiate CMML-like disease.

**RAS pathway mutations drive the enrichment in the mitotic checkpoint kinase PLK1 in pCMML.** To define the mechanism underlying the biological effects of RAS pathway mutations in CMML, transcriptome sequencing (RNA-seq) was performed. We attempted CD34 $+$ selection from PB MNC prior to RNA-seq, however, there was significant attrition in cell numbers resulting in sample inadequacy to carry out large scale experiments. A pilot verification study analyzed RNA-seq data from 5 CMML patients using unsorted PB MNC, 5 using CD14 $+$ sorted PB cells (monocytes) and 5 using CD34 $+$ selected PB progenitor cells (no overlap between groups). This demonstrated a linear correlation in pair-wise gene expression (Supplementary Fig. 4A). Furthermore, comparing expression of housekeeping genes and select genes relevant to myeloid biology in CD34 $+$ sorted and unsorted MNCs revealed no appreciable systematic differences between the two populations for these genes (Supplementary Fig. 4B). Finally, differential gene expression between sorted and unsorted samples identified very few significant differences in the transcriptome (Supplementary Fig. 4C). Taken together, this supports the use of unsorted MNC for transcriptome analysis in our study.

Thus, RNA-seq was performed on PB MNC from 25 CMML patients; 12 pCMML with RAS pathway mutations; and 13 dCMML without RAS pathway mutations. RAS pathway-mutated samples demonstrated a unique gene expression profile with 3729 upregulated and 2658 downregulated genes versus RAS pathway wild-type samples (Fig. 4A, B and Supplementary Data 1). Pathway analysis (ingenuity) identified cascades preferentially...
expressed in RAS pathway-mutated pCMML as (1) mitotic cell cycle process (e.g. WEE1, PLK1, PLK2), (2) cell cycle G2/M checkpoint regulation (e.g. WEE1, AURKA, CHEK1), (3) chromosomal segregation (e.g. CDC45, RPA3), and (4) mitotic cell cycle phase transition (e.g. PLK1; Supplementary Fig. 4D). Focusing on protein coding, targetable genes downstream of the oncogenic RAS pathway, we selected PLK1, a conserved kinase involved in the regulation of cell cycle progression for further mechanistic studies\textsuperscript{16,17}. Although PLK1 was not the top upregulated gene in our data set (Fig. 4C), the rationale for further studying PLK1 included, (a) prior genome-wide RNAi screen that had demonstrated that RAS mutant cells were hypersensitive to PLK1 inhibition\textsuperscript{18}, (b) data demonstrating that RAF1/CRAF localize to the mitotic spindle of proliferating tumor
cells, with RAF1 specifically associating with PLK1 at the centrosomes and spindle poles during G2/Mitosis, (c) the fact that Inhibition of RAF1 phosphorylation impaired PLK1 activation\(^9\), and (d) the PLK1 inhibitor volasertib had already completed extensive pre-clinical and clinical trial testing, with the phase 3 trial in AML providing extensive safety and efficacy data.

Using qPCR on several CMML patient samples, we validated a statistically significant increase in expression of PLK1 in NRAS mutant pCMML \((n = 21)\), in comparison to NRAS wild-type dCMML \((n = 14)\), NRAS mutant dCMML \((n = 4)\), and JAK2 mutant pCMML \((n = 9)\); indicating that overexpression of PLK1 was specific to RAS mutant pCMML. Overexpression of PLK1 was found in CD14 + sorted PB MNC in RAS mutant pCMML relative to RAS wild-type dCMML and normal controls (Supplementary Fig. 4E). Further, we demonstrated a significant positive correlation between PLK1 expression and NRAS\(^{G12D}\) VAF, in NRAS mutant pCMML samples (Fig. 4E). Knockdown of NRAS in NRAS\(^{G12D}\) mutant MNC resulted in reduced PLK1 expression relative to controls (Fig. 4F). Conversely, transfection of NRAS\(^{G12D}\) into RAS wild-type CMML MNC induced upregulation of PLK1 (Fig. 4G). Similar results were obtained with mutant KRAS\(^{G12D}\) (Fig. 4I, H), indicating that this regulation extended to other RAS pathway mutations. These findings were confirmed at the protein level (Supplementary Fig. 4F). We then demonstrated that the association between RAS and PLK1 was clonal RAS mutation specific, by showing that knockdown down of JAK2 in JAK2\(^{V617F}\) mutant/RAS wild-type pCMML MNC, and NRAS in NRAS\(^{G12D}\) mutant dCMML MNC each, had no impact on PLK1 expression (Supplementary Fig. 4G, H)\(^20\). Notably, the mean NRAS VAF in pCMML samples used for these experiments was 38% versus 15% for dCMML samples, suggesting that the differences in mitotic kinase expression with NRAS knockdown may be related to the clonal versus subclonal nature of RAS mutations. Together, these results suggest that acquisition of clonal oncogenic RAS pathway mutations upregulate PLK1; a phenomenon specific to clonal RAS mutant pCMML.

Mutant RAS regulates PLK1 expression through the lysine methyltransferase KMT2A (MLL1). Interrogating molecular events involved in RAS-mediated increase in PLK1 expression, ChIP-seq was performed on BM MNC from 40 CMML patients [RAS pathway-mutated pCMML, \(n = 18\); RAS pathway wild-type dCMML, \(n = 12\)] and healthy, age-matched controls \((n = 10)\). From this data pool we selected 6 samples [3 RAS pathway mutant pCMML (#1 TET2/DNM3A/NRAS, #2 ASXL1/CBL/IDH2/SRSF2 and #3 ASXL1/TET2/EZH2/NRAS] and 3 RAS-pathway wild-type dCMML (#1 TET2/SRSF2, #2 IDH2/SRSF2/ RUNX1 and #3 TET2/SRSF2)] that had adequate number of reads and read depth and that met ENCODE criteria (https://www.encodeproject.org/chip-seq/histone/) for assessment of the three aforementioned histone marks. A peak was defined as either present (MACS2 FDR < 0.05 for a given peak) or not present (MACS2 \(p \geq 0.05\) for a given peak). When we compared the differences between the number of peak bases between RAS mutant pCMML and RAS wild-type dCMML for all three histone marks, there were no statistically significant differences in these six samples (Fig. 5A). However, this analysis does not leverage the confidence of the presence of a given peak across replicates (MACS2 FDR). Therefore, a differential binding analysis (Diff-Bind) was performed, demonstrating numerically more H3K4me1 peaks in pCMML vs dCMML (Fig. 5B). Metagene analysis similarly demonstrated an increase in the number of differentially enriched H3K4me1 peaks in RAS mutant pCMML relative to RAS wild-type dCMML (Supplementary Fig. 5A). Comparisons of overexpressed genes in RAS mutant pCMML with gene promoter enrichment for H3K4me1 peaks demonstrated significant overlap (Fig. 5C and Supplementary Data 2), with PLK1 identified in this group.

Sequence-specific analysis on these six samples also found enrichment of H3K4me1 at the promoter (conservatively defined by a 2Kb region from the transcription start site) of PLK1 (Fig. 5D), with a statistically significant increase in measured H3K4me1 peak height in RAS mutant pCMML vs RAS wild-type dCMML.

We then confirmed these findings with NRAS and KRAS knockdowns that decreased H3K4me1 occupancy at the PLK1 promoter site in NRAS/KRAS mutant pCMML MNC, relative to controls (Fig. 5E, F). Conversely, NRAS\(^{G12D}\)/KRAS\(^{G12D}\) transfection of NRAS/KRAS wild-type dCMML MNC resulted in an increased occupancy of H3K4me1 at PLK1 the promoter (Fig. 5G, H). In contrast, JAK2\(^{V617F}\) knockdown in JAK2 mutant/RAS wild-type pCMML and NRAS\(^{G12D}\) knockdown in NRAS mutant dCMML MNC had no effect on H3K4me1 occupancy (Fig. 5I, J); underscoring the specificity of this axis to clonal RAS pathway

---

**Fig. 3 NRAS\(^{G12D}\) mutation drives a proliferative CMML phenotype.** A. Representative hematopoietic progenitor colony formation assay using NRAS-wild-type CMML patient-derived mononuclear cells (MNCs) after transduction with either a null adenoviral construct (control) or NRAS-expressing vector (NRAS). Red circles indicate individual colonies. Scatter plots depict colony counts at day 12 after inoculation in five individual cases. B Daily cell counts of CMML patient-derived MNCs after siRNA depletion of NRAS in NRAS\(^{G12D}\) cells (above) and transfection of NRAS\(^{G12D}\) in NRAS wild-type cells (below). Representative western blots of three experiments are depicted for validation by assessing phosphorylated ERK (pERK) relative to total ERK (tERK) levels with Vinculin as a loading control. Knockdown of NRAS by qPCR was ≥85%. Indicated \(p\)-value in panels A and B by two-tailed Student’s t test. C-F Receiver operating characteristic curve (ROC) analyses illustrating the diagnostic ability of variant allele frequency (VAF) of NRAS mutations (C) or oncogenic RAS pathway mutations (NRAS, KRAS, CBL, PTEN) (D) at discrimination between pCMML and dCMML phenotypes. If >1 mutation was present in the sample, maximal VAF was used. Impact of VAFRAS as a predictor of pCMML vs dCMML phenotype, using binary RAS mutation status (E), or a continuous VAFRAS value (F). Characterization of the Vav-Cre-Nras\(^{G12D}\) mouse model of CMML is depicted at 6 weeks of age (panels G-I) and when moribund (panels J and K). G Differences in peripheral blood (PB, left) and bone marrow (BM, right) monocytes and neutrophils in Nras\(^{G12D}\) mice relative to wild-type controls. H BM cells were serum- and cytokine-starved for 2 h at 37 °C. Cells were then stimulated with or without 2 ng/ml of mGM-CSF for 10 min at 37 °C. Levels of phosphorylated ERK1/2 (pERK) were measured using phosphor-flow cytometry. Myeloid progenitors are enriched in Lin\(^{hi}\)/Sca1\(^{hi}\)/c-Kit\(^{+}\) cells. Indicated \(p\)-value in panels G and H by two-tailed Student’s t test. I Histopathologic comparisons between wild-type control (top) and Nras\(^{G12D}\) (bottom) mouse PB, BM, and spleen. PB smears depict monocytosis (arrows). BM shows normal hematopoiesis (arrows, above) and megakaryocytic atypia and hyperplasia (arrows, below). Lower power spleen reveals normal white and red pulp architecture (black and red arrows respectively, above) with effacement of this architecture (black and red arrows, below). High power spleen reveals normal white and red pulp architecture (black and red arrows respectively, above) and dysplastic megakaryocytes (arrows, below). J Representative hematopoietic progenitor colony formation assay using MNCs from wild-type control (top) and Nras\(^{G12D}\) (bottom) mice. Bar graph depicts colony counts at day 12 after inoculation. Indicated \(p\)-value by two-tailed Student’s t test. K Kaplan-Meier curve demonstrating overall survival of Nras\(^{G12D}\) mice relative to wild-type controls. Data in panels A, B, G, H and J are presented as mean ± SEM. The indicated \(n\) represents the number of biologic replicates. \(p\)-values are indicated with each comparison. Source data are provided as a Source Data file.
mutations despite JAK2 representing an alternative driver of the pCMML phenotype. Thus, oncogenic RAS signaling enhances expression of PLK1 in association with enrichment of H3K4me1 at its promotor.

Since H3K4me1 is carried out by the lysine methyltransferases KMT2A-D, SET1A and SET1B21, we focused on these entities. RNA-seq analysis detected overexpression of KMT2A (MLL1) and its coactivator MEN1 in RAS mutant pCMML samples versus controls (Fig. 6A), with no statistical difference between RAS mutant pCMML and RAS wild-type dCMML. Unlike in AML, where KMT2A fusions and partial tandem duplications (PTD) are frequent, FISH (fluorescence in situ hybridization) and WES

| Genes | Log2 Fold Change |
|-------|-----------------|
| GLI2  | 5.81            |
| PRMT8 | 3.72            |
| CDK1  | 3.01            |
| PLK4  | 2.31            |
| RAD51 | 2.26            |
| RET   | 2.14            |
| AURKAB| 1.9             |
| WEE1  | 1.4             |
| PLK1  | 1.4             |

E

Relative PLK1 Expression

NRAS VAF

3729 genes up-regulated
2658 genes down-regulated

F

siNT, n=3
siNRAS, n=3

mRNA
(relative expression)

NRAS    PLK1
0 100 200 300 400

G

siNT, n=4
siKRAS, n=4

mRNA
(relative expression)

KRAS    PLK1
0 100 200 300 400

H

siNT, n=4
siKRAS, n=4

mRNA
(relative expression)

KRAS    PLK1
0 100 200 300 400
**Fig. 4** RAS pathway mutations drive expression of mitotic checkpoint kinase **PLK1**. A Unsupervised hierarchical clustering of RNA-seq performed on peripheral blood samples from 35 CMML patients. Cluster 1 (black) with RAS wild-type dCMML cases and Cluster 2 (red) with RAS mutant pCMML cases. B Volcano plot demonstrating differentially upregulated (red) and downregulated (green) genes in RAS mutant pCMML (vs RAS wild-type dCMML) expressed as log2-fold change. Indicated p-values on the y-axis is calculated by the Wald test and false discovery rate-corrected. C Table of most upregulated therapeutically actionable genes in pCMML relative to dCMML. Quantitative PCR (qPCR) validation of **PLK1** expression in patient-derived MNCs from NRAS wild-type (wt) dCMML, NRAS mutant (mt) dCMML, JAK2 mt pCMML and NRAS mt pCMML cases. E Scatter plot comparing relative **PLK1** expression to variant allele frequency (VAF) of NRAS mutation. F qPCR for NRAS and **PLK1** in NRAS mutant pCMML MNCs after transfection with non-targeting siRNA (siNT), or siRNA against NRAS (siNRAS). G qPCR for NRAS and **PLK1** in NRAS wild-type dCMML patient-derived MNCs after transfection with an empty vector (Control) or NRAS<sup>G12D</sup>. H qPCR for KRAS and **PLK1** in KRAS mutant pCMML MNCs after transfection with siNT or siKRAS. I qPCR for KRAS and **PLK1** in KRAS wild-type dCMML MNCs after transfection with an empty vector (Control), or KRAS<sup>G12D</sup>. Western blots in panels F-I are representative of three experiments. Data in panels D and F-I are presented as mean ± SEM. Indicated p-value in panels D-I by two-tailed Student’s t test. The indicated n represents the number of biologic replicates. Source data are provided as a Source Data file.
analyses in 500 CMML samples identified only a single patient with a MLLT3–KMT2A fusion (Fig. 6B), without any KMT2A mutations or PTOR21,22. These data suggest that wild-type KMT2A could drive oncogenic activity in RAS mutant pCMML. ChIP-PCR studies in NRAS mutant pCMML MNC detected occupancy of KMT2A at the PLK1 promoter (Fig. 6C). Knockdown of NRASG12D/KRASG12D in NRAS/KRAS mutant pCMML MNC decreased KMT2A occupancy at the PLK1 promoter relative to controls (Fig. 6D, E). Interestingly, knockdown of NRASG12D in NRASG12D mutant dCMML and JAK2V617F in JAK2V617F mutant pCMML MNC did not result in significant changes in KMT2A occupancy at the PLK1 promoter (Supplementary Fig. 6A, B).

Conversely, transfection of NRAS/KRAS wild-type dCMML MNC with NRASG12D/KRASG12D enhanced KMT2A occupancy at the PLK1 promoter (Fig. 6F, G). Knockdown of KMT2A in NRAS mutant pCMML MNC decreased H3K4me1 enrichment at the PLK1 promoter, correlating with decreased PLK1 expression (Fig. 6H, I). Further, KMT2A knockdown was also associated with a significant decrease in ex vivo cell proliferation in RAS mutant pCMML MNC in comparison to controls (Supplementary Fig. 6C). In addition, inhibition of the KMT2A–MEN1 interaction with Mi-503, an orally bioavailable small molecule inhibitor23, similarly decreased enrichment of H3K4me1 at the PLK1 promoter (Supplementary Fig. 6D). qPCR studies at day 4 after Mi-503 treatment confirmed decreased PLK1 expression (Supplementary Fig. 6E). Since KMT2C and KMT2D are well-known mediators of H3K4me1, we knocked down KMT2C and KMT2D in NRASG12D mutant pCMML MNC but found no changes in H3K4me1 enrichment at the PLK1 promoter versus controls (Supplementary Fig. 6F). These data suggest that KMT2C and KMT2D do not play roles in modulating H3K4me1 in pCMML MNC. Together, these data support the hypothesis that PLK1 expression in clonal RAS mutant CMML is dependent on an unmutated KMT2A as a mediator of oncogenic RAS signaling.

Finally, NRASG12D mutant pCMML–patient-derived xenografts (PDX) using NSG-S (NOD.Cg-Prkdscid Il2rgtm1Wjl/SzJ-SGM3) mice were treated with volasertib. Six-week-old NSG-S mice were sub-lethally radiated with 25 Gy of radiation and 18 h later were injected with 2 ý 10^6 CMML patient-derived BM MNCs via their tail veins (Supplementary Fig. 7A). Four weeks later, after confirming engraftment by documenting hCD45 by flow cytometry, triplicate sets of mice were treated with volasertib 20 mg/kg/week administered intraperitoneally for 2 weeks versus a vehicle control. After 2 weeks of therapy, the volasertib and vehicle-treated mice were sacrificed, followed by flow cytometry and extensive histopathological analysis of blood, BM and spleen specimens (Fig. 7C–H). Immunohistochemistry indicated that neoplastic cells expressed hCD45 by flow cytometry, triplets sets of mice were treated with volasertib 20 mg/kg/week administered intraperitoneally for 2 weeks versus a vehicle control. After 2 weeks of therapy, the volasertib and vehicle-treated mice were sacrificed, followed by flow cytometry and extensive histopathological analysis of blood, BM and spleen specimens (Fig. 7C–H). In addition, partial normalization of splenic follicular architecture (Fig. 7C) and partial restoration of normal hematopoietic islands in BM (Fig. 7D) were observed with volasertib versus vehicle. Findings were confirmed by carrying out flow cytometry with gating strategy depicted in Supplementary Fig. 7B. Analysis demonstrated complete clearance of human hematopoietic component with the pCMML subtype in particular having median OS of <2 years and high rates of AML transformation19. Given limited therapeutic options for affected patients, we carried out this study to define the genetic and epigenetic landscape of pCMML and identify therapies that could modify disease biology. Phenotypically related to pCMML, juvenile myelomonocytic leukemia (JMML) is a pediatric neoplasm often initiated by germline (CBL, NFI, PTPN11) or somatic (NRAS, KRAS, CBL, RRAS) RAS-activating mutations and is described as a bona fide RASopathy26,27. JMML outcome is associated with a dose-dependent effect for RAS pathway activation and accumulation of additional recurrent mutations in genes involved in the polycistronic repressive complex 2 (PRC2–ASXL1) and gene transcription/
signaling (SETBP1 and JAK3). Here, we show in pCMML that acquisition of RAS pathway mutations often occurs early (NRAS\(^{G12D}\) most frequent), commonly on a background of epigenetic (TET2) and splicing gene mutations (SRSF2) and correlates with WHO-defined prognostic factors including leukocytosis, BM blasts and LT to sAML; further implying that severe forms of this disease often seen in the elderly, are also RASopathies that, contrary to JMML, develop on a background of age-related clonal alterations (clonal hematopoiesis). Analysis of mouse models suggest that loss of Tet2, which induces hypermethylation of Spry2 encoding a negative regulator of the RAS signaling pathway, results in a fully penetrant CML phenotype when combined with \(Nra\)^{G12D} expression in mouse hematopoietic cells by synergistically enhancing MAPK signaling. These findings suggest that preexisting mutations in TET2 may favor expansion of cells that acquire a RAS pathway mutation. Of note, hematopoietic progenitor colony hypersensitivity to GM-CSF, an established hallmark of JMML, has also been depicted in CML, especially in RAS mutant pCMML and was also seen in progenitor cells obtained from the Vav-Cre-Nra\(^{G12D}\) pCML mouse model in this study.

WES studies have shown that, on average, CMLL patients harbor between 10–15 somatic mutations per exon/coding region, similar to de novo AML and other myeloid malignancies. Of these 10–15 somatic mutations, a mean number of three affect recurrently mutated genes. These genes (~40 have been identified) largely encode epigenetic regulators (TET2-60%, ASXL1-40%), proteins of pre-mRNA splicing complexes (SRSF2-50%) and members of signaling pathways (NRAS 30%; JAK2 10%). Prior clonal architecture studies at the single cell
Fig. 7 Therapeutic efficacy of targeting PLK1 in pCMML. A Daily cell counts of CMML MNC after transfection with either siNT or siPLK1. Representative western blot depicts validation of PLK1 knockdown from three experiments. Knockdown of PLK1 by qPCR was ≥90%. B Progenitor colony forming assay using CMML MNC with increasing doses of volasertib. Indicated p-value in panels A and B by two-tailed Student’s t test. C, D Histopathologic analysis with H&E staining and immunohistochemistry (IHC) of spleen (C) and BM (D) of murine patient-derived xenografts (PDX) after treatment with vehicle control or volasertib. Magnification is ×200 unless otherwise indicated. hCD45 is human CD45. E Representative flow cytometry of spleen, BM and PB of PDXs after treatment with vehicle control (left) or volasertib (right). The y-axis indicates hCD45 expression status while x-axis indicates murine CD45 (mCD45). Percentages indicate proportion of hCD45+ and mCD45- cells in the respective tissues. F Flow cytometry of proportion of hCD45+ cells in spleen, BM, and PB of PDX mice. Data are presented as mean ± SEM from nine mice. G, H Effect of vehicle versus volasertib treatment on PDX spleen size (G) and weight (H). Data in panel H are presented as mean ± SEM from seven mice treated with vehicle and eight treated with volasertib. Indicated p-value in panels F and H by two-tailed Student’s t test. Data in panels A and B are presented as mean ± SEM. The indicated n represents the number of biologic replicates. p-values are indicated with each comparison. Source data are provided as a Source Data file.
level have reported that in most adult onset hematological malignancies, including CMML, oncogenic RAS pathway mutations are usually secondary/subclonal events3,36. While that is true in dCMML, we show that in pCMML, RAS pathway mutations are often clonal and occur early in the disease course. With the help of a large molecularly annotated database we show that clonal RAS pathway mutations (VAF $\geq 0.19$) were associated with high risk disease features and were associated with an inferior AML-FS (increased risk of LT). Interestingly, NRAS and PTPN11 are also among the recurrently mutated genes associated with LT in patients with myelodysplastic syndromes37. Of note, compared to chronic phase CMML, sAML demonstrated additional somatic mutations in only 44% of cases, indicating that LT can occur without additional driver mutations in the coding regions of DNA. Acquisition of RAS mutations correlated with WBC proliferation and blast cell accumulation, two main prognostic factors recognized by the WHO in CMML, suggesting that RAS mutations form the landscape for epigenetic and evolutionary events leading to AML transformation.

We show that acquisition of clonal RAS pathway mutations in CMML samples is associated with overexpression of PLK1. Polo-like kinases are involved in regulation of cenosome maturation, bipolar spindle formation, anaphase-promoting complex, and chromosome segregation16. Although this was not the top upregulated gene, given the specificity of PLK1 overexpression to clonal RAS mutant pCMML, along with prior work using a genome-wide RNAi screen that had shown sensitivity of RAS mutant cells to PLK1 inhibition and that RAF1/CRAF has shown to have direct interactions (MEK independent) with PLK1 at centrosomes and spindle poles during G2/mitosis, and the fact that phase III trials with PLK1 inhibitors (volaasketib) had already been completed in AML, we chose to further assess the functional and therapeutic relevancy of this mitotic checkpoint kinase18,19. Our ChIP-seq/ChIP-PCR data demonstrated enrichment of H3K4me1, KMT2A, and MEN1 at the PLK1 promoter in RAS mutant pCMML. H3K4me1 is an activating histone configuration associated with increased gene transcription. While the entire KMT2 family (KMT2A-D, SET1A, and SET1B) can result in methylation of H3K4, only KMT2A and KMT2B do so using MEN1 as a coactivator21, KMT2C and KMT2D are reported to be enzymes usually responsible for H3K4 monomethylation, however, we detected no changes in H3K4me1 enrichment at the PLK1 promoter after knockdown of these lysine methyltransferases. Unexpectedly, while KMT2A can cause trimethylation of H3K421, using ChIP-seq, both globally and in a sequence-specific manner, we saw no difference in H3K4me3 enrichment between RAS pathway mutant and wild-type CMML samples, but instead note much higher global and local H3K4me1 at gene promoters in RAS pathway mutant samples. In addition, knockdown of KMT2A and treatment with Mi-503, a small molecule inhibitor of the KMT2A-MEN1 axis, resulted in decreased H3K4me1 promoter occupancy and decreased expression of PLK1. While translocations and PTD of KMT2A have long been implicated in AML pathogenesis, <1% of CMML patients demonstrate KMT2A translocations22 and no patients had KMT2A PTD in our study. Thus, our findings demonstrate that an unmethylated KMT2A can also have an oncogenic role24 and that KMT2A plays an unusual role in pCMML by depositing H3K4me1 at promoters of activated genes.

TET2, ASXL1, and SRSF2 are the most commonly mutated genes in CMML, with truncating ASXL1 mutations being predominantly detrimental20,34. ASXL1 mutations are thought to decrease catalytic activity of PRC2, resulting in depletion of H3K27me3, a repressive mark38-41. A second school of thought attributes detrimental effects of ASXL1 mutations to enhanced activity of the ASXL1-BAP1 complex, resulting in global erasure of H2AK119Ub, and H3K27me3.39 By using ChIP-seq in CMML samples, we demonstrate that H3K27me3 occupancy and recruitment at the PLK1 promoter is not significantly different in RAS pathway-mutated pCMML relative to RAS pathway wild-type dCMML, regardless of ASXL1 mutational status (sequence-specific analysis restricted to PLK1).

PLK1 (volasertib and onvansertib) inhibitors have completed clinical trials for patients with myeloid neoplasms (MDS and AML) with reasonable safety data, prompting us to establish the therapeutic efficacy of these agents in pCMML models22,42-44. While a recent randomized phase III clinical trial testing the efficacy of volasertib with low-dose cytarabine (LDAC vs LDAC and placebo- NCT01721876) in AML patients ineligible for standard induction chemotherapy, did not meet its stated endpoints, this trial was not personalized to subsets of AML patients more likely to respond to therapy. In this study, the overall response rates for volasertib with LDAC vs LDAC with placebo were 25% and 16%, respectively (p = 0.07). We show that targeting PLK1 with volasertib, both in vitro and in vivo, results in effective killing of neoplastic cells, restoring normal hematopoiesis with a differential proliferative advantage favoring RAS mutant pCMML cells. Given these drugs have an established safety record in myeloid malignancies; we aim to translate these findings into an early-phase clinical trial for pCMML.

In summary, we demonstrate acquisition of clonal oncogenic RAS pathway mutations defines a unique subgroup of CMML with poor outcomes. Detection of these mutations offers a therapeutic window of opportunity for pCMML by identifying the significance of KMT2A expression and downstream mitotic check point kinases such as PLK1, which can be targeted efficiently using existing small molecule inhibitors. These findings provide detailed insight into the biology of CMML and lay the foundation for development of personalized clinical therapeutics for this otherwise devastating disease.

**Methods**

**Authorizations, patient cohorts, cell collection, and sorting.** This study was carried out at the Mayo Clinic in Rochester, Minnesota, after approval from the Mayo Clinic Institutional Review Board (IRB- 15-003786), at Gustave Roussy Cancer Center in Villejuif, France, following the approval of the ethical committee Ile-de-France 1 (DC-2014-2091), and at Sigmund Freud University in Vienna, Austria, following the approval of the ethics committee of the City of Vienna (15- 059-VK). Animal experiments were carried out at the Mayo Clinic after approval from the Mayo Clinic IACUC (protocol A00093488-18), the Moffitt Cancer Center in Tampa, Florida (CC- protocol 7140) and the University of Missouri IACUC (protocol M005328). Several patient cohorts were analyzed. In all cases, diagnosis was according to 2016 iteration of the WHO classification of myeloid malignancies1. PB and BM samples were collected in EDTA tubes after informed consent. MNGs were collected on Ficoll-Hypaque whereas CD14- and CD34-positive cells were sorted using magnetic beads and the AutoMacs system (Miltenyi Biotech, Bergisch Gladbach, Germany). In five cases, bicalon mucosa cells were used as germine control cells. All international cohorts on which NGS was performed have been represented in Supplementary Table 1 including the Mayo Clinic, Austrian and French (GFM) cohorts. Of the 591 patients with CMML collected in chronic phase and in a random orientation, which was screened in 397 using a 36-gene panel targeted NGS assay. The 592 patients with CMML recruited in France (n = 417) and in Austria (n = 175) had molecular information obtained using a 38-gene NGS panel. One patient cohort on which WES was performed is represented in Supplementary Table 3. This included 48 patients with CMML whose paired MNC samples were collected in chronic phase and in LT to perform WES. A second cohort of 18 patients with CMML was used to perform WES on samples serially collected along disease progression. This cohort had been described previously6. All patient samples were obtained either at diagnosis, or at first referral, which was usually within 3-6 months of diagnosis. Patients not meeting these criteria were not included in these cohorts. All patients were treatment naive at the time of collection of their samples for sequencing. None of them were in a clinical trial at the time of sample collection. The disease stage is mentioned in the table under the World Health Organization-2016 diagnosis section. While 250 of the Mayo Clinic patients were eventually treated with hypomethylating agents, given the retrospective nature of this study and treatment at centers outside of Mayo Clinic, accurate response data and duration of treatment information is not available. The same applies to the European cohorts. All patients in this data set that underwent
allogeneic stem cell transplant (10%) were accurately accounted for and were censored in survival data calculations, as transplant is the only disease modifying treatment and eligibility for NCC.

The following statistical methods were used on the clinical data sets: Distribution of continuous variables was statistically compared using Mann–Whitney or Kruskal–Wallis tests, while nominal or categorical variables were compared using the Chi-Square or Fischer’s exact test. Time to event analyses used the method of Kaplan–Meier for univariate comparisons using the log-rank test. Overall survival was calculated from the date of diagnosis to date of death or last follow-up, while AML-free survival was calculated from date of diagnosis to date of AML transformation or death. All statistical calculations were carried out using JMP statistical software, version 16.0, SAS Institute Inc. Cary, NC, 1989-2019.

**Whole exome sequencing.** One megabase of genomic DNA was sheared with the Covaris S2 system (Beverly, MA). A protocol for library preparation was performed using Agilent SureSelect Human All Exon V5 (Agilent Genomics, Santa Clara, CA), according to the manufacturer’s instructions. The final libraries were indexed, pooled, and paired-end sequenced (2 x 100 bp) on an Illumina HiSeq platform (San Diego, CA). Germ-line DNA was obtained from CD3-positive cells for 5 patients of the CMML/sAML dataset and for 17 patients with CMML with serial WES analyses. Since CD3-positive cells are known to be contaminated with tumor cells we performed MuTect2 in the ‘tumor-only’ mode [47]. For all identified mutations and indels variant allele frequencies (VAF) in the corresponding CD3-positive DNA was retrieved from BAM files. All the variants with p values from Fisher exact test below 10^-4 were considered as somatic. Regional copy number variation was determined using the ‘tumor-normal’ model. This analysis was performed using Agilent SureSelect Human All Exon V5 (Agilent Genomics, Santa Clara, CA) with indexed paired-end adapters (NEXTBio). Allele frequency in the normal population was calculated using the Exome Aggregation Consortium ExAC database ([http://exac.broadinstitute.org](http://exac.broadinstitute.org)). Alternative alleles with <5 reads and/or a frequency <5% were excluded. All variants passing the sequencing quality-control filters and within a gene coding region, regardless of database population frequency, were considered as somatic.

Hematopoietic progenitor colony formation assay. PB and/or BM samples were first treated with ammonium chloride to deplete red blood cells, washed in RPMI, then plated at a final concentration of 5 x 10^5 – 10^6 cells/mL into methylcellulose formulated with recombinant cytokines to support the optimal growth of erythroid progenitors (BFU-E and CD34+ cells). Colony counts were performed using a microscope. Colony-forming units (CFU) were divided into erythroid (CFU-E), granulocyte (CFU-G), monocyte (CFU-M), and mixed lineage (CFU-GM). Colony-forming units (CFU-G) and mixed lineage (CFU-GM) were considered putative drivers if reported in the COSMIC v89 database. Only stop-gain, splice-loss, and splicing-inducing mutations were considered to be putative drivers in tumor suppressor genes. SCNA analysis was performed with the python and computational software package pandas. The python and computational software package pandas. The python and computational software package pandas. The python and computational software package pandas.
Chromatin immunoprecipitation and sequencing. Approximately 100,000 cells from each CMLM patient were used for native chromatin immunoprecipitation (N-ChIP). Cells were washed in ice for 20 min in lysis buffer containing 0.1% Triton X-100, 0.1% deoxycholate, and protease inhibitors. Extracted chromatin was digested with 90 U of MNase enzyme (New England Biolabs) for 6 min at 25°C. The reaction was quenched with 250 μL of EDTA post-digestion. A mix of 1% Triton X-100 and 1% deoxycholate was added to digested samples and incubated on ice for 20 min to precipitate the antibody-DNA complex. Following an overnight 4°C incubation, samples were washed twice with Low Salt Buffer (20 mM Tris-HCl [pH 8.0], 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 150 mM NaCl) and twice with High Salt Buffer (20 mM Tris-HCl [pH 8.0], 0.1% SDS, 1% Triton X-100, 150 mM EDTA, and 500 mM NaCl). DNA–antibody complexes were eluted in Elution Buffer (100 mM NaHCO3, 1% SDS) incubated at 65°C for 90 min. Protein digestion was performed on the eluted DNA samples at 30°C for 30 min (Qiagen Protease mix). ChIP DNA was purified using Sera-Mag beads (Fisher Scientific) with 30% PEG before library construction. Library construction using enriched DNA from histone mark ChIP and Input was made according to a modified indexed paired-end library protocol (Illumina Inc., USA). Unindexed libraries were prepared for each sample and epigenetic mark. Samples were pooled at 1:1:2:2 ratios for H3K4me1/H3K4me3: H3K27me3: Input, respectively and subjected to paired-end sequencing (100 cycles on Illumina Hiseq2500). Sequencing to a depth of ~25 million (H3K4me1 and H3K4me3) or ~30 million reads (H3K27me3 and Input) per sample was performed. Reads were multiplexed and aligned to the human genome (GRCh38/hg38) using bowtie2. Adjusted reads were sorted and indexed using samtools and areas in the genome enriched with aligned reads (also called peaks) were called using MACS227 callpeak v2.2.6 with the following parameters: --format BAM --peakshift 1000 --qvalue 0.1. For broad-cutoff 0.1 for H3K4me1, MACS2 peak calls were considered relative to their respective input controls using the paired end mode at a default q-value cut-off of 0.05 as indicated. Both H3K4me1 and H3K27me3 samples were called using default options for broad peaks whereas H3K4me3 samples were called using default options for narrow peaks according to ENCODE recommendation. With peak comparisons, for each group, overlapping peaks were identified by Homer mergePeaks v4.11.1 software with the following options: --gsize hs -d given. Regions of differential enrichment were derived using the R Bioconductor package DiffBind (citation: DiffBind: differential binding analysis of ChIP-Seq peak data, http://bioconductor.org/packages/release/bioc/vignettes/DiffBind/inst/doc/DiffBind.pdf) and areas of interest were annotated using Homer. To identify differences in DNA binding, the Bioconductor package DiffBind v2.16.0 was used in R v4.0.3. Reads were counted in intervals using dba.count function and mapQChc = 1, minOverlap = 3 parameters. Differential binding affinity analysis was performed using dba.analyze with method = OMA - D = P - F - p values. Differentially bound sites identified were then used to plot MA with dba.plotMA function. Bigwig files, normalized for sequencing depths, have been generated with deepTools. Individual tracks for dyssaptic and proliferative samples were visualized using UCSC repository. ChIP-seq data that support the findings in Figure 5 have been deposited in GEO under series GSE158377.

Chromatin immunoprecipitation-qPCR. ChIP was performed as described previously. Briefly, PB MNC from CMLM patients (20 × 106) were crosslinked with 1% formaldehyde directly into the medium for 10 min at room temperature. The cells were then washed with PBS, collected by centrifugation at 800× g for 5 min at 4°C and re-suspended in Cell Lysis Buffer (20 mM Tris pH 8.0, 150 mM NaCl, 0.5% NP-40), and incubated on ice for 15 min. Resulting pellets were then re-suspended in Nuclear Lysis Buffer (50 mM Tris [pH 8.1], 10 mM EDTA; 0.5% KCl; 0.5% NP-40), and incubated on ice for 15 min. Resulting pellets were then re-suspended in Nuclear Lysis Buffer (20 mM Tris [pH 8.0], 10 mM EDTA; 1% SDS) and chromatin was sheared to an average fragment size of 200–600 bp using a water bath sonicator (Bioruptor, Diagenode, Denville, NJ). Input chromatin was separated from the individual sample prior to purification of amplified products for qPCR analysis. Chromatin was incubated overnight at 4°C with magnetic beads (Dynabeads Protein G, Invitrogen) and the following antibodies (4 μg each): KMT2A (Abcam); H3K4me1 (Abcam); normal rabbit IgG (EMD Millipore). Following immunoprecipitation, crosslinks were removed and immunoprecipitated DNA was purified using spin columns and subsequently amplified by qPCR. Quantitative PCR was performed using a primer set to amplify a region located between -588bp and -482bp relative to the first base pair of exon one of PLK1. Jaccard similarity indices were then calculated by subtracting the IgG control Ct from the immunoprecipitated Ct. Fold enrichment was then calculated as 2^ΔΔCt.

Electroporation technique for siRNA experiments. Patient-derived PB and/or BM MNC were first treated with ammonium chloride to deplete mature, non-nucleated cell types and then stained with 0.05% 7-AAD (Sigma-Aldrich) against BRD4 and nucleophosmin (see Hematopoietic progenitor colony forming assays for further details) to allow for progenitor expansion. Cells were labeled from methylcellulose by solubilizing them with PBS warmed to 37°C. Cells were washed with RPMI before being re-suspended in Lonza electroporation solution from Kit C (Lonza, Basel, Switzerland) and pre-cleared with protein W (Lonza, Basel, Switzerland). For every one million cells transfected, 2 μg of plasmid constructs or 60 pg of siRNA was used. Electroporation efficiency was monitored by transfecting an eGFP construct as a control. After electroporation, cells were maintained in RPMI supplemented with StemSpan (StemCell Technologies #02691) for 72 h before experiments were conducted.

Recombinant adenoaviral transduction procedure. We utilized eGFP Adenovirus (#11060) and NRAS viral oncogene homolog adenovirus (#11565), both from Vector Bios (Malvern, PA) for the transduction procedure. Briefly, we aliquoted 10 million PBMC cells from CMLM patients into 1 mL of RPMI and added either control (GFP) or NRAS-expressing adenoviruses at an MOI of 100. We spun the cells for 30 min at 1200 RPM and let the cells sit an additional 30 min at room temperature. We then collected the cells and plated them at the same concentration (using RPMI) into cytokine enriched methylcellulose media for colony formation (MethoCultTMGF H4434, StemCell Technologies, Vancouver, CA). After 7 days at 37°C, we enumerated and imaged the progenitor colony growth.

In vitro drug studies on progenitor colonies with PLK1 inhibitor. Patient-derived PB and/or BM MNC were plated at 4 × 10⁶ cells per well in 96-well plates in 200 μL of RPMI supplemented with 10% fetal bovine serum and 10X StemSpan (StemCell Technologies #02691). Media was also supplemented with either DMSO as a vehicle control. Volasertib (PLK1 inhibitor) was tested at concentra-
tions 10 nM, 100 nM, 250 nM, 500 nM, and 1000 nM. After 72 h, the cells were centrifuged to aspirate and replace media. Drug assays were completed on day seven. At this time, each well was counted using a hemocytometer. Subsequently, an MTS assay was performed following the manufac-
turer’s protocol.

Western blot. PVDF membranes were blocked in Tris-buffered saline and 0.3% Tween-20 (TBST) with 5% skim milk overnight at 4°C. Primary antibodies used in western blotting were prepared in a solution of TBST with 2% skim milk at a working concentration of 1:1000 and included antibodies against PLK1 (4355), KMT2A (14197), phospho-ERK1/2 (Thr202/Tyr204), JAK2 (3230), and STAT3 (4904) all from Cell Signaling Technology. Antibodies were also used against vinculin (Bethyl, A302-535A), total ERK1/2 (R&D, MAB1576), NRAS (Santa Cruz, Sc-31), RRBP-conjugated goat-anti-rabbit IgG (Millipore, 12-348) and goat-anti-
mouse IgG (Millipore, 12-349) secondary antibodies were used at a concentration of 1:5000 and detected using SuperSignal West Dura Extended Duration Substrate (Thermo Scientific, 34076).

Genetically engineered mouse models. The NRAS(12D2) genetically engineered mouse model used for this manuscript was developed in collaboration with the Zhang laboratory at the University of Wisconsin in Madison. All mouse lines were maintained in a pure C57BL/6 genetic background (>N10). Mice bearing a conditional oncogenic Nras allele (NrasLox-stop-Lox (LSX) G12D(+/-)) were crossed to Vav-Cre mice. Two-week-old mice were harvested by cervical dislocation, and genotypes were analyzed. To identify differences in gene expression, the Bioconductor package Diffbind v2.16.0 was used in R v4.0.3. Reads were counted in intervals using dba.count function and mapQChc = 1, minOverlap = 3 parameters. Differential binding affinity analysis was performed using dba.analyze with method = OMA - D = P - F - p values. Differentially bound sites identified were then used to plot MA with dba.plotMA function. Bigwig files, normalized for sequencing depths, have been generated with deepTools. Individual tracks for dysplastic and proliferative samples were visualized using UCSC repository. ChIP-seq data that support the findings in Figure 5 have been deposited in GEO under series GSE158377.

Patient-derived xenograft models. The NOD.Cg-Pkd<sup>cre</sup>/Ld<sub>2rg</sub>x<sub>1Wj</sub>S6-GS3MG3 (NSG-S) mice were used for the PDX experiments. The mice were 6-8 weeks old and maintained in a pure C57BL/6 genetic background. Mice were maintained on a 24 h light/24 h dark cycle with 2.5 Gy with a cesium source irradiator and xenografted 24 h after irradiation, with x10 (6) BM MNCs from NRAS mutant CMLM patients. Informed consent was
2 weeks. Mice were monitored daily for signs of disease and euthanized by intraperitoneal injection of pentobarbital sodium. Pooled samples were analyzed on a LSRII flow cytometer (BD Biosciences). Cytopenias, cutaneous and gastrointestinal toxicity, and adverse events were noted throughout the treatment period. All statistical calculations were carried out using the R statistical software package (version 3.6.1). Variables were compared using the Mann-Whitney U test. OS was calculated from the date of diagnosis to date of death or last follow-up, with death used as a competing risk. The impact of therapy on the neoplastic clone (assessed by immunohistochemistry) was compared using the chi-square test. To this point, findings of this study have been deposited in the Marmalade consortia data repository (https://marmalade-consortia.org/). The endnotes of this article have been deposited in the Marmalade consortia data repository (https://marmalade-consortia.org/).

**Data availability**

We do not have patient consent to release raw NGS data. Any inquiries for accessing these data should be directed to Minna Pattnaik (pattnaik.minna@mayo.edu) and we will grant access to the de-identified datasets for research purposes. WES data that supports the findings in Figs. 4 and 5 have been deposited in EGA under the study ID EGAD00001007026. RNA-seq data that supports the findings in Figs. 4 and 5 have been deposited in GEO under the accession number GSE156289. The ChIP-seq data that supports the findings of this study have been deposited in GEO under the accession number GSE156377. The UCSC genome browser tracks can be accessed through the UCSC genome browser (https://genome.ucsc.edu/; db/mhuber/CMLM_RAS_H3K4me1). Source data are provided with this paper.

**References**

1. Arber, D. A. et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127, 2391–2405 (2016).
2. Pattnaik, M. M. & Tetteri, A. Chronic myelomonocytic leukemia: 2018 update on diagnosis, risk stratification and management. Am. J. Hematol. 93, 824–840 (2018).
3. de Witte, T. et al. Allogeneic hematopoietic stem cell transplantation for MDS and CML: recommendations from an international expert panel. Blood 129, 1753–1762 (2017).
4. Sharma, P. et al. Allogeneic hematopoietic stem cell transplantation in adult patients with myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) overlap syndromes. Leuk. Lymphoma 58, 872–881 (2017).
5. Coston, T. et al. Suboptimal response rates to hypomethylating agent therapy in chronic myelomonocytic leukemia: a single institutional study of 121 patients. Am. J. Hematol. 94, 767–779 (2019).
6. Merlevede, J. et al. Mutation allele burden remains unchanged in chronic myelomonocytic leukemia responding to hypomethylating agents. Nat. Commun. 7, 10076 (2016).
7. Pattnaik, M. M. et al. Blast phase chronic myelomonocytic leukemia: Mayo-MDACC collaborative study of 171 cases. Leukemia 32, 2512–2518 (2018a).
8. Elena, C. et al. Integrating clinical features and genetic lesions in the risk assessment of patients with chronic myelomonocytic leukemia. Blood 128, 1408–1417 (2016).
9. Itoyokson, R. et al. Prognostic score including gene mutations in chronic myelomonocytic leukemia. J. Clin. Oncol. 31, 2428–2436 (2013a).
10. Pattnaik, M. M. et al. ASXL1 and SETBP1 mutations and their prognostic contribution in chronic myelomonocytic leukemia: a two-center study of 466 patients. Leukemia 28, 2206–2212 (2014).
11. Rici, C. et al. RAS mutations contribute to evolution of chronic myelomonocytic leukemia to the proliferative variant. Clin. Cancer Res. 16, 2244–2250 (2010).
12. Pylayeva-Gupta, Y., Grabocka, E. & Bar-Sagi, D. RAS oncogenes: weaving a tumorigenic web. Nat. Rev. Cancer 11, 761–774 (2011).
13. Joseph, C. et al. Deciphering hematopoietic stem cells in their niches: a critical appraisal of genetic models, lineage tracing, and imaging strategies. Cell Stem Cell 13, 520–533 (2013).
14. Li, Q. et al. Hematopoiesis and leukemogenesis in mice expressing oncogenic NrasG12D from the endogenous locus. Blood 117, 2022–2032 (2011).
15. Padron, E. et al. GM-CSF-dependent pSTAT5 sensitivity is a feature with therapeutic potential in chronic myelomonocytic leukemia. Blood 121, 5068–5077 (2013).
16. Liu, Z., Sun, Q. & Wang, X. PLK1, a potential target for cancer therapy. Sci. Transl. Med. 10, 22–33 (2016).
17. Matheson, C. J., Backos, D. S. & Reigan, P. Targeting Wee1 kinase in cancer. Trends Pharmacol. Sci. 37, 872–881 (2016).
18. Luo, J. et al. A genome-wide RNA screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell 137, 835–848 (2009).
19. Mielgo, A. et al. A MEK-independent role for CRAF in mitosis and tumor progression. Nat. Med. 17, 1641–1645 (2011).
20. Pattnaik, M. M. et al. (2019). Clinical correlates, prognostic impact and survival outcomes in chronic myelomonocytic leukemia patients with the JAK2V617F mutation. Haematologica 104, e236–e239 (2019).
21. Kernougou, C. et al. KMT2A and KMT2B mediate memory function by affecting distinct genomic regions. Cell Rep. 20, 538–548 (2017).
22. Dohner, H. et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 129, 424–447 (2017).
23. Pattnaik, M. M. et al. Therapy related-chronic myelomonocytic leukemia (CMMiL): Molecular, cytogenetic, and clinical distinctions from de novo CMMiL. Am. J. Hematol. 90, 65–73 (2015b).
24. Borkin, D. et al. Pharmacologic inhibition of the Menin-MLL interaction blocks progression of MLL leukemia in vivo. Cell Rep. 31, 1641–1645 (2017).
25. Yoshimi, A. et al. Robust patient-derived xenografts of MDS/MPN overlap syndromes capture the unique characteristics of CMML and JMML. Blood 138, 355–369 (2016).
26. Stiegitz, E. et al. The genomic landscape of juvenile myelomonocytic leukemia. Nat. Genet. 47, 1326–1333 (2015a).
27. Stiegitz, E. et al. Subclonal mutations in SETBP1 confer a poor prognosis in juvenile myelomonocytic leukemia. Blood 125, 516–524 (2015b).

Received: 24 January 2020; Accepted: 20 April 2021;
Published online: 18 May 2021
29. Caye, A. et al. Juvenile myelomonocytic leukemia displays mutations in components of the RAS pathway and the PRC2 network. Nat. Genet. 47, 1324–1331 (2015).

30. Mason, C. C. et al. Age-related mutations and chronic myelomonocytic leukemia. Leukemia 30, 906–913 (2016).

31. Kunimoto, H. et al. Cooperative epigenetic remodeling by TET2 loss and NRAS mutation drives myeloid transformation and MEK inhibitor sensitivity. Cancer Cell 33, 44–59 (2018).

32. Patnaik, M. M. et al. Phase 1 study of lenzilumab, a recombinant anti-human GM-CSF antibody, for chronic myelomonoctytic leukemia (CMML). Blood https://doi.org/10.1182/blood2019043532 (2020).

33. Ball, M., List, A. F. & Padron, E. When clinical heterogeneity exceeds genetic heterogeneity: thinking outside the genomic box in chronic myelomonocytic leukemia. Blood Cancer J. 6, e385 (2016).

34. Patnaik, M. M. & Tefferi, A. Cytogenetic and molecular abnormalities in chronic myelomonocytic leukemia. Blood Cancer J. 6, e393 (2016).

35. Itzykson, R. et al. Cytogenetic architecture of chronic myelomonocytic leukemia. Blood 121, 2186–2198 (2013b).

36. Makishima, H. et al. Dynamics of clonal evolution in myelodysplastic syndromes. Nat. Genet. 49, 204–212 (2017).

37. Abdel-Wahab, O. et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. Cancer Cell 22, 180–193 (2012).

38. Abdel-Wahab, O. & Levine, R. L. Mutations in epigenetic modifiers in the pathogenesis and therapy of acute myeloid leukemia. Blood 121, 3563–3572 (2013).

39. Balasubramani, A. et al. Cancer-associated ASXL1 mutations may act as gain-of-function mutations of the ASXL1-BAP1 complex. Nat. Commun. 6, 7307 (2015).

40. Uni, M. et al. Modeling ASXL1 mutation revealed impaired hematopoiesis caused by derepression of p16INK4a through aberrant PRC1-mediated histone modification. Leukemia 33, 191–204 (2019).

41. Ottmann, O. G. et al. Phase I dose-escalation trial investigating volasertib as monotherapy or in combination with cytarabine in patients with relapsed/refractory acute myeloid leukemia. Br. J. Haematol. 184, 1018–1021 (2018).

42. Caldwell, J. T., Edwards, H., Buck, S. A., Ge, Y. & Taub, J. W. Targeting the wee1 kinase for treatment of pediatric Down syndrome acute myeloid leukemia. Pediatr. Blood Cancer 61, 1767–1773 (2014).

43. Janning, M. & Fiedler, W. Volasertib for the treatment of acute myeloid leukaemia: a review of preclinical and clinical development. Future Oncol. 10, 1157–1165 (2014).

44. Shen, R. & Seshan, V. E. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. Nucleic Acids Res. 44, e131 (2016).

45. Patnaik, M. M. et al. DNMT3A mutations are associated with inferior overall and leukemia-free survival in chronic myelomonocytic leukemia. Am. J. Hematol. 92, 56–61 (2017).

46. Pardanani, A. et al. Extending Jak2V617F and MPLW515 mutations analysis to single hematopoietic colonies and B and T lymphocytes. Stem Cells 25, 2358–2361 (2007).

47. Kalari, K. R. et al. MAP-MSI35: mayo analysis pipeline for RNA sequencing. BMC Bioinform. 15, 224 (2014).

48. Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efeficient and robust pipeline for assigning sequence reads to genomic features. Bioinformatics 30, 923–930 (2014).

49. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140 (2010).

50. Kim, D., Langmead, B. & Salzberg, S. L. HISAT2: fast and memory-efficient alignment for next-generation sequencing data. Nat. Methods 9, 357–358 (2012).

51. Li, H. et al. 1000 Genome Project Data Processing Subgroup. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079 (2009).

52. Zhang, Y. et al. Model-based analysis of ChIP-seq (MACS). Genome Biol. 9, R137 (2008).

53. Ramirez, F. et al. deepTools2: a next generation web server for deep-seq data analysis. Nucleic Acids Res. 44, W160–W165 (2016).

54. Kent, W. J. et al. The human genome browser at UCSC. Genome Res. 12, 996–1006 (2002).

55. Coma, A. et al. Nuclear factor of activated T cells-dependent down-regulation of the transcription factor gloma-associated protein 1 (GL1) underlies the growth inhibitory properties of arachidonic acid. J. Biol. Chem. 291, 1933–1947 (2016).

56. Li, H. & Durbin, R. Fast and accurate read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760 (2009).

57. Bernstein, B. E. et al. The NIH roadmap epigenomics mapping consortium. Nat. Biotechnol. 28, 1045–1048 (2010).

58. Dammermann, A. et al. Kras is required for adult hematopoiesis. Stem Cells 34, 1859–1871 (2016).

59. Abdel-Wahab, O. et al. Deletion of ASXL1 results in myelodysplasia and severe in vivo developmental defects. J. Exp. Med. 210, 2641–2659 (2013).

60. Wang, J. et al. Endogenous oncogenic NRAS mutation initiates hematopoietic malignancies in a dose- and cell type-dependent manner. Blood 118, 368–379 (2011).

61. You, X. et al. Unique dependence on Sos1 in Kras (G12D)-induced leukemogenesis. Blood 132, 2575–2579 (2018).

62. Gjertsen, B. T. & Schoffski, P. Discovery and development of the Polo-like kinase inhibitor volasertib in cancer therapy. Leukemia 29, 11–15 (2015).

63. Rudolph, D. et al. Efficacy and mechanism of action of volasertib, a potent and selective inhibitor of Polo-like kinases, in preclinical models of acute myeloid leukemia. J. Pharmacol. Exp. Ther. 352, 579–589 (2015).

Acknowledgements

Current publication is supported in part by grants from the “Gerstner Family Career Development Award”, the “Center for Individualized Medicine- Mayo Clinic”, and “The Henry J. Predolin Foundation for Research in Leukemia, Mayo Clinic, Rochester, MN, USA”. This publication was also supported by CTSA Grant Number KL2 TR000136 from the National Center for Advancing Translational Science (NCATS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. R.M.C. was supported by the 2020 Eagles 5th District Cancer Telethon Cancer Research Fund Fellowship Award. M.E.F.P. was supported by C1A36526. S.N. was supported by Foundation ARC 2017, Foundation Gustave Roussy and Swiss Cancer League (grant FKC-3985-08-2016) grants. ES group is labeled “Equipe labellisée de la Ligue Nationale contre le Cancer” and supported by the French National Cancer Institute. P.V. was supported by the Austrian National Science Foundation (FWF) grants F4704-B20 and P30625-B28. A.A.M was supported by the Conquer Cancer Foundation of American Society of Clinical Oncology (ASCO) Young Investigator Award (YIA) and Grant. We would like to thank the University of Wisconsin Carbone Comprehensive Cancer Center (UWCCC) for use of its Shared Services (Flow Cytometry Laboratory, Genome Editing and Animal Models Shared Resource, and Experimental Pathology Laboratory) to complete this research. This work was supported by R01CA152108 and a Scholar Award from the Leukemia & Lymphoma Society to J.Z. This work was also supported in part by NIH/NCI P30 CA041520-11-UW Comprehensive Cancer Center Support. The diagram in Supplementary Fig. 7A was created using BioRender.com.

Author contributions

R.M.C., D.V., T.L., D.L.M., I.H., A.B., A.A.M., S.N., A.Y., I.P., G.C., S.L.S., J.H., and M.M.P. performed experiments, analyzed sequencing data, and helped write the manuscript. A.V., X.Y., M.B., E.P., and J.Z. helped perform mouse experiments with T.W., S.H.K., G.S., P.V., E.S., T.G., K.G., and L.L.A. A.B., A.A.M., P.V., and M.M.P. performed experiments, analyzed sequencing data, and helped write the manuscript. A.V., X.Y., M.B., E.P., and J.Z. helped perform mouse experiments with A.V., M.E.B., and E.P. conducting PDX experiments and X.Y. and J.Z. developing the GEMM. B.A., K.D.R., M.N., C.P., K.B., and M.E.F. helped with ChIP-seq. M.T.H. helped with histopathology and flow cytometry. T.W., S.H.K., G.S., P.V., E.S., T.G., K.G., and M.M.P. helped write and edit the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-021-23186-w.

Correspondence and requests for materials should be addressed to S.N. or M.M.P.

Peer review information Nature Communications thanks Gautam Borthakur and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
