Published in final edited form as:

Leukemia. 2017 October ; 31(10): 2228–2234. doi:10.1038/leu.2017.51.

AMKL chimeric transcription factors are potent inducers of leukemia

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Abstract

Acute megakaryoblastic leukemia in patients without Down syndrome is a rare malignancy with a poor prognosis. RNA sequencing of fourteen pediatric cases previously identified novel fusion transcripts that are predicted to be pathologic including *CBFA2T3-GLIS2*, *GATA2-HOXA9*, *MN1-FLI1*, and *NIPBL-HOXB9*. In contrast to *CBFA2T3-GLIS2* which is insufficient to induce leukemia, we demonstrate that the introduction of *GATA2-HOXA9*, *MN1-FLI1* or *NIPBL-HOXB9* into murine bone marrow induces overt disease in syngeneic transplant models. With the exception of *MN1*, full penetrance was not achieved through the introduction of fusion partner genes alone, suggesting that the chimeric transcripts possess a unique gain of function phenotype. Leukemias were found to exhibit elements of the megakaryocyte erythroid progenitor (MEP) gene expression program, as well as unique leukemia-specific signatures that contribute to transformation. Comprehensive genomic analyses of resultant murine tumors revealed few cooperating mutations confirming the strength of the fusion genes and their role as pathologic drivers. These models are critical for both the understanding of the biology of disease as well as providing a tool for the identification of effective therapeutic agents in preclinical studies.

INTRODUCTION

Acute megakaryoblastic leukemia (AMKL) accounts for ~10% of childhood acute myeloid leukemia (AML). Clinical outcomes are variable, ranging from greater than 80% to less than 40% in patients with or without Down syndrome respectively(1–6). Genomically, Down syndrome AMKL is characterized by a founding *GATA1* mutation that cooperates with

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

trisomy 21, followed by the acquisition of additional somatic mutations in epigenetic and kinase signaling genes(7–9). To gain insight into the genetic etiology of AMKL patients without Down syndrome, we previously performed transcriptome sequencing on diagnostic blasts from a discovery cohort of 14 pediatric cases and substantiated our findings in a recurrence/validation cohort. This analysis confirmed the unique biology of this disease as evidenced by novel fusion transcripts including *CBFA2T3-GLIS2*, *GATA2-HOXA9*, *MN1-FLI1*, and *NIPBL-HOXB9*(10).

To confirm their role in oncogenesis and gain insight into the mechanism whereby these novel fusions promote disease, we introduced each of them as well as their individual gene counterparts into murine hematopoietic progenitor cells that were assessed in murine syngeneic transplantation models. Accompanying histologic, immunophenotypic, and genome-wide molecular characterization of resultant tumors delineated common and unique modes of leukemogenesis.

MATERIALS AND METHODS

Nucleotide Sequencing

RNA and DNA library construction for transcriptome and whole exome DNA sequencing (WES) were done as per manufacturer's instructions using the Illumina True-seq RNA sample preparation V2 and Agilent SureSelect MOUSE XT2 all exon 50Mb kits. Sequencing was completed on the Illumina HiSeq 2000. Analysis of data which includes mapping, coverage, quality assessment, SNV/Indel detection, and prediction of deleterious effects of missense mutations have been described previously(10–12). Please see supplementary methods for details regarding use of transcriptome sequencing data in gene expression analyses.

Murine Bone Marrow Transplantation and Colony Forming Assays

All experiments involving mice were reviewed and approved by the Institutional Animal Care and Use Committee. Bone marrow from 4–6 week old female C57BL/6 mice was harvested, lineage depleted, and cultured in the presence of cytokines for 24 hours prior to transduction on RetroNectin (Takara Bio Inc.). Ecotropic envelope pseudotyped retroviral vectors were produced as previously described(13). For colony forming assays, cells were harvested 48 hours following transduction, sorted for vector-encoded mCherry or GFP expression, and plated on methylcellulose containing cytokines (Stem Cell technologies, Vancouver, BC). Colonies were counted after 7 days of growth at 37°C, harvested and serially replated. For primary bone marrow transplants, 4–8×10⁶ unsorted cells were injected intravenously on day 0. An aliquot of cells was retained to determine transduction efficiencies and dosage of fusion protein-positive cells per mouse (Supplementary Methods). Recipient mice were conditioned with total body irradiation at a dose of 1100 rads divided on days –1 and 0. For secondary transplants, 3–5×10⁶ splenic cells from moribund mice were isolated and injected intravenously into recipient mice conditioned with 600 rads.

Flow Cytometry

Cells were stained with antibodies for 15 minutes at 4°C, washed, and resuspended in 4',6-diamidino-2-phenylindole containing solution (1µg/ml DAPI) for analysis using FACS LSR II D (BD Biosciences). Sorting was done on a FACS Aria III (BD Biosciences). For a list of antibodies used, please see supplementary methods.

Histology

Tissues were fixed in 10% buffered formalin, embedded in paraffin, and sections stained with hematoxylin and eosin.

Array-based comparative genomic hybridization of mouse tumors

The Mouse Genome CGH 244K microarray (G4415A; Agilent, Santa Clara, CA) was customized as previously described(14, 15). Array hybridization was performed according to the manufacturer's recommended protocols. The circular binary segmentation algorithm implemented in the DNACopy package from Bioconductor was applied to the normalized log2 ratio data to identify copy number alterations (CNA) for each tumor sample(16, 17).

RESULTS

AMKL Chimeric Transcripts Enhance Cellular Self Renewal and Induce Leukemogenesis

Approximately 50% of AML cases carry cytogenetic aberrations leading to fusion genes, many of which promote oncogenesis through the acquisition of enhanced cellular self-renewal(18). To evaluate the effects of the *CBFA2T3-GLIS2*, *GATA2-HOXA9*, *MN1-FLII*, and *NIPBL-HOXB9* gene fusions, we introduced each of them into murine hematopoietic cells and assessed their effect on *in vitro* colony replating (Supplementary Figures S1,2). Expression of each of the fusion genes resulted in a marked increase in self-renewal capacity, with colony formation persisting through ten replatings. Immunophenotypic analysis revealed evidence of megakaryocytic differentiation in *CBFA2T3-GLIS2* and *MN1-FLII* cohorts, whereas *NIPBL-HOXB9* and *GATA2-HOXA9* cells expressed myeloid progenitor markers (Supplementary Figure S3). Transplantation of fusion gene-modified bone marrow cells into syngeneic recipients induced overt leukemia in all cohorts with the exception of *CBFA2T3-GLIS2*, suggesting that there is an essential requirement for cooperative mutation(s) in cases expressing this chimeric gene (Figure 1A). At the time of clinical illness, blood counts revealed leukocytosis, anemia, and thrombocytopenia (Supplementary Figure S4). Necropsy of moribund mice revealed neoplastic infiltrates throughout all organs assessed including the peripheral blood, bone marrow, spleen, lymph nodes, liver, CNS, kidneys, and lungs confirming the systemic nature of the disease (Figure 1B and Supplementary Table S1). Tumor cells with megakaryocytic features by morphology were present at a low frequency in *NIPBL-HOXB9* bone marrow sections, in contrast to *MN1-FLII* leukemias that were uniformly megakaryocytic (Figure 1B). Although *GATA1-HOXA9* leukemias did not demonstrate megakaryocytic morphology, immature and dysplastic megakaryocytes were interspersed throughout the bone marrow, a finding that is absent in classical AML mouse models such as those driven by *PML-RARA* and *MLL-AF9* fusion genes (Figure 1B)(19, 20). Consistent with immunophenotyping of CFUs, *MN1-FLII*

tumors demonstrated the presence of megakaryocytic markers whereas *NIPBL-HOXB9* and *GATA2-HOXA9* cells carried markers consistent with myeloid progenitors (Supplementary Figure S5). Secondary transplants of all leukemias generated disease with shorter latencies than those observed in the primary transplant setting (Supplementary Figure S6), implying selection for more aggressive leukemic clones. We conclude that *MNI-FLII*, *NIPBL-HOXB9*, and *GATA2-HOXA9* induce AML with megakaryocytic features in mice.

Fusion Driven Leukemias Carry Cooperating Mutations

To characterize the tumors at the molecular level, five primary leukemic samples carrying each of the three fusions were subjected to array comparative genomic hybridization (aCGH) and WES (Figure 2A,B Supplementary Figures S7,8 and Supplementary Tables S2,3). Samples demonstrated a small number of cooperating mutations with 1.53 ± 0.42 CNAs (range 0–6) and 5.7 ± 1.04 non-silent single nucleotide variations (SNVs)/small insertion deletions (Indels) (range 2–18) per case (Supplementary Table S4). Overall, cases carried an average of 7.27 ± 1.09 non-silent mutations (range 2–18). Of the non-silent SNVs/Indels, only 2.33 ± 0.36 were expressed at the RNA level (range 1–5) and 1.2 ± 0.28 (range 0–3) of these were predicted to have a functional consequence (Supplementary Table S4). Thus the number of cooperating mutations in these tumors is extremely low, supporting the strength of the fusion genes in driving leukemogenesis. Despite this, recurrently mutated genes were identified. These include activating mutations in *Flt3*, *Kras*, and *cMet*, as well as loss of function mutations in the tumor suppressors *Phactr4*, *Wt1*, and *Tet2* (Supplementary Figures S7,8). A comparison between fusion subtypes did not reveal any statistically significant differences in mutational frequency between the tumors induced by the three fusion genes (Supplementary Table S4).

Transcriptome Sequencing Identifies Myeloid Progenitor Signatures and Megakaryocytic Features

Transcriptome sequencing of tumor cohorts, along with 19 normal hematopoietic progenitor subsets spanning myeloid differentiation, confirmed unique gene expression patterns between normal and leukemic cells induced by each of the fusions (Supplementary Figures S9,10 and Supplementary Methods). Gene set enrichment analysis (GSEA) of the leukemia signatures against the 19 normal hematopoietic progenitor subsets was consistent with immunophenotyping of the tumors. Specifically, *MNI-FLII* tumors were significantly enriched for the megakaryocyte progenitor signature while *NIPBL-HOXB9* and *GATA2-HOXA9* were enriched for common myeloid progenitor (CMP) and monocyte precursor signatures respectively (Supplementary Figure S11). Although immunophenotyping and GSEA of *NIPBL-HOXB9* and *GATA2-HOXA9* tumors suggested a lack of megakaryocytic features, our histologic assessment demonstrated these tumors to have dysplastic and immature megakaryocytes present. Therefore we identified differentially expressed genes in megakaryocyte precursor subsets (MegDEGs) compared to other hematopoietic subsets and determined expression levels of these genes in our tumor populations (Table 1). *MNI-FLII* leukemias shared the greatest similarity to megakaryocyte precursors, with elevated expression levels of 31–71% of MegDEGs. Despite the lack of megakaryocytic cell surface markers in *NIPBL-HOXB9* and *GATA2-HOXA9* tumors, these leukemias also shared 14–31% and 15–24% upregulated MegDEGs respectively. To confirm that this enrichment was

specific, we repeated this analysis using differentially expressed genes in polychromatic erythroblasts and found no enrichment in our leukemia subsets. The megakaryocyte erythroid progenitor subset (MEP) of DEGs was most consistently shared with all fusions, ranging from 22–44% (Table 1). Tjissen and colleagues previously described a megakaryocytic transcriptional network defined by five transcription factors including GATA1, GATA2, RUNX1, FLI1, and TAL1 whose combinatorial pattern dictates target gene expression(21). To determine if the expression pattern of these five factors in our leukemia subtypes contributes to the specific megakaryocytic genes upregulated, we first evaluated their RNA levels when compared to normal hematopoietic subsets (Figure 3A). *NIPBL-HOXB9* and *GATA2-HOXA9* leukemias were characterized by elevated *RUNX1* and *FLI1* expression, whereas *MNI-FLI1* leukemias highly expressed all five factors. Consistent with this hypothesis, we found megakaryocytic target genes upregulated by *RUNX1* and *FLI1* binding to be enriched in *NIPBL-HOXB9* and *GATA2-HOXA9* leukemias whereas *MNI-FLI1* leukemias demonstrated enrichment for genes bound by all combinations of the five transcription factors (Figure 3B). We conclude that all three fusion genes invoke component(s) of the megakaryocytic gene expression program to varying degrees.

Myeloid leukemias have been shown to invoke hematopoietic progenitor gene expression programs. An unsupervised analysis of fusion gene-driven leukemias along with hematopoietic stem cell (HSC), CMP, granulocyte monocyte progenitor (GMP), and MEP subsets revealed that our murine leukemias have a greater similarity to committed myeloid progenitors than to HSCs (Supplementary Figure S12A). *K*-means clustering identified elements of the GMP and MEP signatures in all three subtypes (Supplementary Figure S12B). Despite the closer relationship at the gene expression level to committed myeloid progenitors, elements of the HSC program could still be identified in all three subtypes (Supplementary Figure S12B).

All three fusion constructs contain DNA binding domains of genes known to play a role in malignancy. To determine if known targets of these genes were uniquely upregulated in the corresponding leukemias, genes differentially upregulated between the three subtypes were identified for GSEA. Accordingly, gene sets derived from hematopoietic cells transduced with *EWSR1-FLI1*, *HOXA9* plus *MEIS1*, or *GATA2* were significantly enriched in the *MNI-FLI1* and *GATA2-HOXA9* subsets respectively (Supplementary Figure S13A)(22–24). As both NIPBL and HOXB9 proteins play a role in binding to regulatory regions of genes we extracted a unique gene set derived from genome wide NIPBL ChIP sequencing data in embryonic stem cells(25). Greater than 6000 genomic loci were bound by NIPBL in this dataset. 479 of these loci were expressed in our leukemias and contained HOXB9 consensus binding sites as determined by MATCH™ analysis (Supplementary Figure S13B and Table S5). Upregulation of these 479 gene targets was highly enriched in *NIPBL-HOXB9* leukemias in contrast to NIPBL targets that lacked a strong HOXB9 consensus binding site, demonstrating cooperativity between these proteins in targeting the fusion to genomic loci ($P=0.006$ vs. $P=0.249$). Interestingly, the top leading edge genes in this analysis revealed the oncogenes *cMyc* and *Myb*, as well as several Ras-associated guanine exchange factors (GEFs) and GTPases suggesting that the NIPBL-HOXB9 fusion protein is able to upregulate expression of canonical AML oncogenic pathways (Supplementary Figure S13B). We conclude that leukemias driven by *MNI-FLI1*, *GATA2-HOXA9*, and *NIPBL-HOXB9* carry

components of megakaryocytic and myeloid progenitor gene expression signatures consistent with their differentiation block/self-renewal phenotype seen in CFU assays as well as common and unique leukemia-specific gene expression signatures.

Fusion Genes Have a Unique Function Independent of their Individual Counterparts

A common characteristic between all three fusions is the presence of protein interaction domains contributed by the N-terminal partner, and DNA binding domains contributed by the C-terminal moiety (Supplementary Figure S1). To determine if these fusions have a novel gain of function distinct from their individual counterparts, we introduced each partner gene into murine bone marrow cells for CFU and transplantation experiments. *MNI*, *HOXA9* and *HOXB9* genes all conferred the ability of transduced cells to serially replat, whereas *FLI1* and *GATA2* genes did not (Supplementary Figure S14). Similarly, introduction of the *NIPBL* exons 1–6 (*NIPBL*^{ex1–6}), the portion present in the fusion gene, failed to allow cells to serially replat (Supplementary Figure S14). The introduction of *MNI* into hematopoietic cells led to a highly penetrant leukemia as has been shown previously (Figure 4)(26–28). In contrast *MNI*, *HOXA9*, *HOXB9*, and *FLI1* all had >75% disease-free survival with few myeloid leukemias resulting from their over expression, while *GATA2* and *NIPBL* failed to induce any disease at all (Figure 4). Thus only *MNI* overexpression is sufficient to induce reproducible disease whereas other subgroups require both fusion partners for leukemogenesis.

NIPBL-HOXB9 Requires Interaction with MAU2 to Induce Leukemogenesis

NIPBL has not been previously implicated in malignancy and introduction of the N-terminal portion failed to allow serial replating or induce leukemia. Therefore, to evaluate the role of *NIPBL* in our model we introduced two different SNVs previously shown to disrupt binding of *NIPBL* to the cohesin component *MAU2* (G15R, P29Q)(29). Surprisingly, these single alterations abrogated the ability of cells carrying the fusion to serially replat as well as to induce leukemia (Supplementary Figure S15A–B). To verify interaction of the fusion with *Mau2*, we introduced the *NIPBL-HOXB9* wild type and mutant fusion constructs into NIH3T3 cells. *Mau2* co-immunoprecipitated with wild type but not mutant fusion constructs confirming this interaction (Supplementary Figure S16). Introduction of the *Mau2* shRNA into leukemia cells transformed by *NIPBL-HOXB9*, followed by their *in vitro* culture resulted in extinction of cells lacking *Mau2* expression in contrast to cells transduced with an empty vector or ineffective shRNAs, thereby demonstrating a strong selection bias against downregulation of *Mau2* (Supplementary Figure S17A). Furthermore, transplantation of flow sorted *NIPBL-HOXB9* leukemia cells expressing sh*Mau2* into sublethally irradiated recipients resulted in a trend towards prolonged survival compared to controls ($P=0.04$ Supplementary Figure S17B). Analysis of the peripheral blood from mice that succumbed to leukemia in the *Mau2* shRNA cohort revealed a loss of the knockdown construct with >60% of the blood consisting of single positive *NIPBL-HOXB9* leukemia cells consistent with our *in vitro* results (Supplementary Figure S18). We conclude that *NIPBL* plays a critical role in *NIPBL-HOXB9* driven leukemogenesis, in part mediated by its interaction with *Mau2*, the component of the cohesin complex that recruits *NIPBL* to the genome.

DISCUSSION

Acute myeloid leukemia is frequently driven by cytogenetic aberrations that lead to gene fusion events, the most common of which include *MLL* gene rearrangements, *AML1-ETO*, *CBFB-MYH11*, and *PML-RARA*. Despite evidence that these fusions are key genetic drivers of the malignancy, some fail to induce leukemia in murine models in the absence of a secondary event such as mutations driven by N-ethyl-N-nitrosourea (ENU), or *in vitro* manipulation with culture and cytokine support to select susceptible progenitor populations prior to transplantation(30, 31). Although overexpression of *ERG* in a syngeneic transplant setting leads to megakaryoblastic leukemia, only one murine model based on genomic mutations that occur in patients with non-Down syndrome AMKL currently exists: that which models the *RBM15-MKL1* fusion seen in infant AMKL(32, 33). In this knock-in model, while all mice demonstrated evidence of hematopoietic dysregulation, only two out of 15 mice developed leukemia after a latency of greater than 18 months.

We sought to establish additional and more efficient murine models for AMKL by evaluating four previously described fusion products identified in human cases by RNAseq(10). *NIPBL-HOXB9*, *GATA2-HOXA9*, and *MN1-FLI1* all induced acute leukemia with high penetrance and an average latency of 143 ± 76 days (range 47–435) (see supplementary methods). Surprisingly we saw few cooperating mutations by WES and aCGH supporting the strength of their leukemic potential. Although all of the murine tumors analyzed had at least one additional mutation, a significant number of these are likely passenger mutations because they are not recurrent, are not predicted to have functional consequences affecting the encoded protein, and fail to affect genes previously shown or predicted to play roles in malignancy (Supplementary Table S3).

Of the three fusion genes, leukemias induced by *MN1-FLI1* exhibited the strongest canonical megakaryocytic phenotype in the majority of mice (Figure 1B, and Supplementary Figure S5). These tumors also manifested the strongest megakaryocyte progenitor gene expression signature (Figure 3A–B, and Supplementary Figures S11,12A–B). This phenotype is not surprising, given the known role of FLI1 in megakaryopoiesis. Binding sites for FLI1 are present on enhancers of megakaryocyte-specific genes, and cooperative binding with GATA1 mediates synergistic expression(34). Upregulation of FLI1 targets, however, is likely not the sole reason for this phenotype, as expression of *EWSR1-FLI1* in hematopoietic cells leads to an erythroid leukemia(22). Furthermore, *MN1* alone led to a megakaryocytic phenotype in a significant proportion of mice and evidence suggests the N-terminal portion of MN1 plays a role in megakaryocyte differentiation (Supplementary Figure S19)(28). Other groups have previously introduced the *MN1* gene into murine bone marrow for syngeneic transplant models and did not see a propensity for megakaryocytic disease(26–28). This difference may reflect transplant methodologies, as we did not use 5-fluorouracil (5-FU) to eliminate mature progenitor cells in the bone marrow, choosing instead to lineage deplete un-stimulated marrow and limit *in vitro* cell culture to 72 hours prior to transplantation. Thus the population of cells transduced with the oncogene in our study and those of others is likely non-identical.

In contrast to *MNI-FLII*, the gene partners of the *NIPBL-HOXB9* and *GATA2-HOXA9* fusions do not suggest an obvious propensity toward AMKL. Despite this, we saw elements of the MEP gene expression program in both subtypes, a finding that is absent in *MLL-AF9* driven leukemia(35). Additionally, tumor cells with megakaryocytic features were present upon histologic analysis of *NIPBL-HOXB9* leukemias while *GATA1-HOXA9* mice demonstrated abundant immature and dysplastic megakaryocytes interspersed amongst the tumor cells. Thus these models both demonstrate features of megakaryoblastic disease albeit not to the extent of *MNI-FLII*. Both *NIPBL-HOXB9* and *GATA2-HOXA9* fusions demonstrated a gene expression program consistent with up-regulation of HOX target genes. The role of HOX genes in AML has been well established and thus the resultant murine leukemias that carry a predominantly myeloid phenotype are perhaps not unexpected(36). RNAseq analysis of a large pediatric AML cohort has not yet been published, thus it may be that these fusions exist in non-AMKL disease and are not specific to the AMKL subtype *per se*. Nonetheless, we have identified additional cases of *NIPBL-HOXB9* and *GATA1-HOXA9* in an expanded pediatric AMKL cohort by RNAseq, strengthening their association with this disease(37).

Similar to their frequency in human hematopoietic malignancies, *Flt3* and *Kras* activating mutations were identified in 20% and 13% of mouse leukemias respectively (Supplementary Figure S8 and Tables S2, S3)(38, 39). The identification of these mutations serve to not only validate the applicability of our mouse models in studying human disease, but also provide direct functional evidence of their role in the progression of disease(40). Studies in humans and mice have shown that FLT3 plays an important role in AML, either through mutational activation or upregulation by chimeric oncogenes(41, 42). *NIPBL-HOXB9*, *GATA2-HOXA9*, and *MNI-FLII* leukemias all up-regulated *Flt3* when compared to normal myeloid progenitors (Supplementary Figure S12C, Table S6). The promoter/enhancer region of *Flt3* contains ETS, HOX and GATA binding sites, providing a rationale for the ability of the three different fusion genes to uniformly increase *Flt3* expression(43, 44).

In conclusion, we have generated a highly penetrant AMKL mouse model and two AML mouse models with megakaryocytic features, all of which have been characterized phenotypically and at the genomic level. Mechanistic studies have demonstrated that these chimeric oncogenes block maturation and enhance self-renewal through the upregulation of progenitor gene expression programs. With the exception of *MNI*, both fusion gene partners were required for full penetrance of disease. Cooperating mutations identified by next generation sequencing were significant for activating kinase mutations that have previously been shown to confer cytokine independence. In summary, these models replicate many features of human AMKL, as evidenced by their clinical presentation and molecular characteristics. They provide valuable tools for future biologic and therapeutic studies to further our understanding and improve outcomes of this rare and devastating subtype of leukemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank the staff of Flow Cytometry Core, Veterinary Pathology, as well as the Hartwell Center for Biotechnology and Bioinformatics of St Jude Children's Research Hospital. Jinghui Zhang and the department of computational biology graciously provided previously established bioinformatic pipelines and support. We thank Charles J. Sherr, Thomas A. Look, and Jeffery M. Klco for comments on the manuscript. This work was supported by grants from the Eric Trump Foundation, Gabrielle Angel Foundation, and the American Lebanese Syrian Associated Charities of St Jude Children's Research Hospital.

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Figure 1. AMKL Fusion Genes Lead to a Highly Penetrant Leukemia

(A) Murine bone marrow was transduced with a control retroviral construct (MIC) or one of four fusion genes (*CBFA2T3-GLIS2*, *NIPBL-HOXB9*, *GATA2-HOXA9*, or *MN1-FLI1*) followed by injection into lethally irradiated recipients. Leukemia free survival is shown. (B) Bone marrow sections from a representative mouse in each of the cohorts are shown. White arrows: neoplastic cells with megakaryocytic features, Yellow arrows: immature megakaryocytes, Red arrows: dysplastic megakaryocytes.

Figure 2. Cooperative Mutations in Fusion Driven Leukemias

(A) Cumulative SNVs/Indels as determined by WES in each of the subgroups. Total number of non-silent mutations are shown, in addition to those whose gene products are expressed as determined by RNAseq. Mutations were analyzed by PROVEAN prediction algorithm to assess their functional consequence. (B) Total number of cooperating mutations per tumor subtype as determined by aCGH and WES.

Figure 3. Hematopoietic Regulators Invoke Megakaryocytic Gene Expression Signatures
(A) Expression levels in murine leukemias of five key transcription factors in megakaryocytes: *RUNX1*, *FLI1*, *TAL1*, *GATA1*, and *GATA2*. MEP: megakaryocyte erythroid progenitor; PRE: proerythroblast; BPE: basophilic erythroblast; OCE: orthochromic erythroblast; MkP: megakaryocyte progenitor; iMk: immature megakaryocyte; MMk: mature megakaryocyte (B) Single sample GSEA was done for leukemias induced by each of the three fusions for target genes of the 6 occupancy patterns in megakaryoblasts as described by Tijssen and colleagues (21, 45).

Figure 4. Overexpression of Fusion Gene Partners is Insufficient to Induce a Highly Penetrant Leukemia with the Exception of MN1

(A) *MN1*, *FLI1*, *GATA2*, *HOXA9*, *NIPBL^{e1-6}*, and *HOXB9* transduced bone marrow was injected into lethally irradiated recipients. Leukemia free survival is shown. Due to size restraints, the *NIPBL^{e1-6}* retroviral construct carries NIPBL exons 1–6, the portion of the gene involved in the fusion.

Percentage of Differentially Upregulated Progenitor Subset Genes Highly Expressed in Fusion Driven Leukemias

Table 1

Progenitor Subset ¹	#Genes ²	FDR ²	NIPBL-HOXB9	GATA2-HOXA9	MNI-FLI1
MEP	651	0.02	30.72%	21.97%	43.78%
MkP	570	0.1	21.23%	15.26%	70.18%
iMk	202	0.2	14.36%	24.26%	30.69%
MMk	372	0.1	15.05%	21.24%	55.65%
PCE	778	0.0001	1.41%	2.31%	2.57%

¹ Megakaryocyte erythroid progenitor (MEP); Megakaryocyte progenitor (MkP); Immature megakaryocyte (iMk); Mature megakaryocyte (MMk); Polychromatic erythroblast (PCE). See supplementary methods for immunophenotypic definition of subsets.

² Number of genes differentially upregulated in hematopoietic subset; False discovery rate (FDR) cut-off for gene list