Published in final edited form as:

Exp Hematol. 2008 May ; 36(5): 523–532. doi:10.1016/j.exphem.2007.12.018.

Meeting Report: Seventh International Workshop on Molecular Aspects of Myeloid Stem Cell Development and Leukemia, Annapolis, May 13–16, 2007

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The seventh biennial workshop on Molecular Aspects of Myeloid Stem Cell Development and Leukemia was held again in Annapolis, Maryland, May 13–16, 2007. There were nearly 50 oral presentations from leading scientists and approximately 100 participants attending all together. Clearly, interest in this biennial meeting, which started in 1995, continues to be strong. This was again a forum where researchers had the opportunity to present their work in an informal atmosphere in the lovely historic coastal town of Annapolis. It was held at the Loews Annapolis Hotel and was organized by Dr. Linda Wolff (National Cancer Institute, Bethesda, MD, USA), and Drs. Steven Ackerman and Giuseppina Nucifora (University of Illinois at Chicago, Chicago, IL, USA). There were many reports in the areas of, molecular regulation of differentiation, delineation of molecular mechanisms of leukemogenesis, and mouse models for acute myeloid leukemia (AML) which represented major advances since the previous meetings. Of particular interest this year, however, was a new topic on clonal disorders, which included presentations on myelodysplastic syndrome (MDS) and myeloproliferative disease (MPD). There has been only a limited amount of research on this subject, but investigations into the cause of these diseases have been increasing in recent years. Some reasons for this are the following. First, the number of people affected by these disorders out numbers those with AML. Second, these disorders, which are characterized by imbalances in differentiation, can lead to death and third, in some patients they can lead to AML. We were pleased to introduce talks on epigenetic regulation in myeloid development and leukemia and on normal and leukemic stem cells. Since the demonstration of leukemia-initiating cells with the self-renewal properties of stem cells in 1997, there has been an intense interest in determining the characteristics of these cells and implications which can be exploited for therapy^{1–3}.

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Regulation of lineage specification

The first two sessions of the workshop were devoted to presentations concerning normal myeloid cell development. Myelopoiesis begins with hematopoietic stem cells (HSCs). These can be found in the aortic-gonadal-mesonephros (AGM) region of the embryo during definitive hematopoiesis. They expand there and migrate to the fetal liver and spleen. Finally, before birth HSCs migrate to the bone marrow (BM) where hematopoiesis is sustained during adult life. HSCs are capable of maturing into all myeloid lineages (see Figure 1) by first committing to the common myeloid progenitor (CMP), and then to either the megakaryocyte/erythroid progenitor (MEP) or the granulocyte macrophage progenitor (GMP). A number of key transcription factors have been identified as being instructive in determining the commitment of these progenitors, and these are indicated in Figure 1. It should be noted, however, as described in more detail below, that many other transcription factors contribute to the establishment and maintenance of cell fate (see ref ⁴).

The zebrafish model has developed into a powerful tool for the analysis of hematopoiesis (reviewed in ⁵). Large-scale forward screens performed over the last ten years have resulted in numerous mutants that have contributed to our understanding of many aspects of myelopoiesis. The equivalent of the AGM region in the zebrafish is also found in the dorsal aorta. Around 4–5 days after fertilization the location of blood formation moves to the kidney. Dr. P.P. Liu (National Human Genome Research Institute, NIH, Bethesda) had previously demonstrated that *gata1* plays an essential role in zebrafish hematopoiesis, showing significant conservation of function between mammals and zebrafish ⁶. Using mutants of *gata1* he reported at the workshop that partial *gata1* activity is sufficient for definitive hematopoiesis in the kidney, but not primitive hematopoiesis, which occurs in the intermediate cell mass (ICM) located ventral to the notochord in the trunk. In addition, he discovered that there are two waves of definitive hematopoiesis, and *runx1* is absolutely required for the first one.

Historically, the transcription factor PU.1 has been the subject of intense study because it is essential for myeloid development and dysregulation of its function was shown to lead to leukemia ^{7,8}. It has been demonstrated that a high concentration of the transcription factor PU.1 promotes macrophage development, while a low concentration promotes B cell development *in vitro* ⁹. This idea has been tested directly using mice with inducible alleles of the gene (*Sfpi1*) that encodes PU.1. Dr. R.P. DeKoter (University of Cincinnati College of Medicine, Cincinnati, OH) showed that in mice homozygous for a novel hypomorphic allele of the *Sfpi1* gene, in which PU.1 expression is ~20% of normal levels, B cell development is profoundly blocked. Myeloid development in these mice is impaired during fetal hematopoiesis, but after birth a dramatic expansion of immature myeloid cells occurs. These results suggest that a high rather than low concentration of PU.1 is required for generation of B cell progenitors. Furthermore, PU.1 functions to limit proliferation of immature myeloid cells during adult hematopoiesis.

Dr. D.G. Tenen (Harvard Institutes of Medicine, Boston, MA) reported an important leap in our understanding of how genes such as PU.1 could be tightly regulated. He showed that phylogenetically conserved elements participate in the initiation of antisense transcription

and the antisense RNAs function as important modulators of proper dosages of PU.1. Distal and proximal cis-regulatory elements controlling PU.1 expression interact in a nucleoprotein complex. Interestingly, the same complex is also formed between elements utilized to generate antisense RNAs. Their results indicate that there is an evolutionarily conserved regulatory mechanism that exploits a shared transcription factor complex and chromatin configuration for biogenesis of sense and antisense RNAs.

Other speakers addressed the role of PU.1 in specifying differentiation at the macrophage/neutrophil bifurcation. It has been suggested that the relative levels of PU.1 and C/EBP α specify macrophage versus neutrophil lineage choice¹⁰. Work presented by Dr. H. Singh (Howard Hughes Medical Institute, University of Chicago, IL) and R. Dahl (University of New Mexico, Albuquerque, NM) established a regulatory circuit comprised of counter antagonistic repressors Egr-1,2/Nab-2 and Gfi-1, which are downstream of PU.1 and C/EBP α respectively. Dr. Singh showed that PU.1 induces Egr-2 and Nab-2 which repress neutrophil genes during macrophage differentiation. Using *PU.1*^{-/-} progenitors, he and his colleagues demonstrated that at sub-threshold levels, this Ets transcription factor regulates a mixed pattern (macrophage/neutrophil) of gene expression, whereas increased PU.1 levels refine the pattern and promote macrophage differentiation by modifying the counter antagonistic repressors, Egr-1,2/Nab-2 and Gfi-1¹¹. Dr. Dahl provided evidence that C/EBP α , on the other hand, induces Gfi-1, which physically interacts with PU.1, repressing PU.1-dependent transcription and PU.1-induced macrophage differentiation¹².

Since Gfi1 is a central player in transcriptional programs controlling neutrophil development, it is not surprising that mutations in Gfi1 are associated with human Severe Congenital Neutropenia (SCN), leading to recurrent bacterial and fungal infections. While mutations in ELA2 predominate in SCN, mutation of Ela2 in mice does not recapitulate SCN. Since Gfi1 regulates ELA2 and genetic deletion of Gfi1 results in murine neutropenia, Dr. H.L. Grimes (Cincinnati Children's Hospital Medical Center, Cincinnati, OH) has set out to determine whether human SCN-associated GFI1N382S mutations are causal in SCN. His lab examined the consequences of GFI1N382S expression in primary murine hematopoietic cells and found that the GFI1N382S mutation inhibits DNA binding and results in a dominant negative block to murine granulopoiesis. Moreover, Gfi1N382S selectively derepresses the monopoietic cytokine CSF1 and its receptor. Overall, their data show that GFI1 functions as a rate-limiting granulopoietic molecular switch and this is relevant to the pathogenesis of SCN.

A great deal of attention at the workshop over the years has been focused on the mechanisms by which transcription factors direct lineage choice. An important aspect of positive and negative regulation of transcription factor activity lies in post-translational phosphorylation events. Dr. M. Buitenhuis (University Medical Center Utrecht, Utrecht, The Netherlands) reported that protein kinase B (PKB)/GSK-3 mediated regulation of C/EBP α modulates granulopoiesis. This signaling pathway abrogates the inhibitory phosphorylation of C/EBP α . Expression of constitutively active Glycogen Synthase Kinase-3 was shown to reduce neutrophil development and induce eosinophil differentiation. On the other hand, ectopic expression of a mutant C/EBP that cannot be phosphorylated by PKB/GSK-3 dramatically reduced eosinophil differentiation and induced neutrophil development.

C/EBP regulation of myeloid lineage specification was also addressed by Dr. S. J. Ackerman (University of Illinois at Chicago, Chicago, IL), whose laboratory is studying the combinatorial activities of the C/EBP ϵ activator and repressor isoforms in granulocyte differentiation. Human C/EBP ϵ is expressed as four distinct isoforms (32, 30, 27, 14kD) through alternative promoters, RNA splicing, and translational start sites. The C/EBP $\epsilon^{32/30}$ isoforms are transcriptional activators, whereas this group has reported that C/EBP ϵ^{27} is a potent repressor of GATA-1 in vitro¹³, and C/EBP ϵ^{14} , which contains only DNA binding and bZIP but no transactivation domain, functions as a dominant negative repressor of C/EBP ϵ^{32} and other C/EBPs. Dr. Ackerman reported on the activities of these isoforms in reprogramming differentiation of cord blood CD34+ HSCs transduced with retroviral IRES-EGFP expression vectors. The C/EBP ϵ^{32} activator isoform strongly supported eosinophil differentiation over neutrophil (G-CSF-induced) or erythroid (EPO-induced) development, with >90% eosinophil colony formation (even in the absence of IL-5), whereas C/EBP ϵ^{27} strongly inhibited eosinophil differentiation, consistent with its predicted role as a repressor of GATA-1, which is required for eosinophilopoiesis. In contrast, C/EBP ϵ^{14} favored erythroid at the expense of eosinophil development, strongly inhibiting IL-5-induced eosinophil differentiation, consistent with it acting as a repressor of other C/EBPs required for myeloid development.

A surprising finding in the area of lineage choice regulation was that a negative regulator of the cell cycle, *Ink4b*(p15), when deleted in mice causes alterations in the differentiation potential of the common myeloid progenitor (CMP). Dr. L. Wolff (National Cancer Institute, NIH, Bethesda, MD) presented data that *Ink4b* null mice have an increase in granulocyte-macrophage progenitors (GMPs) in the bone marrow. Furthermore, differentiation of purified CMP from these mice in vitro and in vivo resulted in increased myeloid potential (increased CFU-GM) and decreased erythroid potential (decreased BFU-E)¹⁴. These results could not be explained by alterations in cell cycle frequency or self-renewing potential.

Other regulators of myeloid cell development

A presentation with emphasis on the role of signaling in myelopoiesis was presented by Dr. S. Collins (Fred Hutchinson Cancer Research Center, Seattle, WA). He and his colleagues found that Ca⁺⁺ signaling, previously largely unexplored, regulates the proliferation and differentiation myeloid leukemia cells. The activated form of CaMKII γ is frequently present in leukemia cells but the activity of the enzyme is markedly diminished when these cells undergo terminal differentiation. In myeloid cells that differentiate in response to all-trans retinoic acid (ATRA), they found that CaMKII γ phosphorylates the retinoic acid receptor and inhibits its transcriptional activity by enhancing its interaction with the transcriptional corepressor N-CoR. A pharmacological inhibitor of CaMKII enhances RAR α transcriptional activity and terminal differentiation in response to ATRA. In addition, it inhibits proliferation of other types of myeloid cells. Importantly, their data indicate that CAMKII is a critical regulator of multiple downstream signal transduction pathways involved in regulating proliferation and differentiation of myeloid leukemia cells including the MAP kinase, JAKStat and GSK3 β / β -catenin pathways.

The predominant Src kinase in myeloid cells that is activated upon G-CSF stimulation is Lyn, and the laboratory of Dr. S.J. Corey (M.D. Anderson Cancer Center, Houston, TX) has discovered how the cytokine receptor activates this kinase. Their data suggest that Gab2 forms a complex with Lyn and following G-CSF stimulation, Gab2 recruits Shp2, which dephosphorylates Lyn pTyr507, leading to Lyn activation.

The *Ets*-related transcription factor GABP has been shown previously by Dr. A.G. Rosmarin (Brown University, Providence, RI) and colleagues to regulate expression of CD18 and other myeloid genes that are important for innate immunity ¹⁵. At the meeting he reported that Gabp appears to be required for proliferation of myeloid progenitors. Bone marrow from floxed *Gabpa*/Mx1-Cre mice, injected with polyI-C, exhibit a marked reduction in myeloid colony-forming units. GABP appears to be required for proliferation because his lab has been able to show that Gabpa-null mouse embryonic fibroblasts (MEFs) exhibit a profound defect in S-phase entry ¹⁶. Interestingly, Gabpa is necessary and sufficient for quiescent MEFs to re-enter the cell cycle and GABP seems to regulate a pathway that is independent of the canonical cyclinD/CDK/Rb/E2F pathway.

Physiological stress can have an impact on myelopoiesis. Drs. B. Hoffman and D.A. Lieberman (Fels Institute of Cancer Research and Molecular Biology, Philadelphia, PA) pointed out that Gadd45 proteins have important functions in regulating myeloid development in response to physiological stress. Gadd45a^{-/-} mice and gadd45b^{-/-} mice display compromised myeloid differentiation and increased apoptosis in vitro following acute stimulation with a variety of differentiating cytokines. Furthermore, the recovery of the bone marrow myeloid compartment following 5-fluorouracil-induced myeloablation was much slower in the deficient mice.

New genomic approaches to the analysis of hematopoietic gene expression are highly likely to provide new insights into development. Dr. S.M. Weissman's group at Yale University (New Haven, CT) has been employing genome tiling arrays for the analysis of patterns of gene expression, transcription factor binding, and chromatin modifications in cultured lines of myeloid cells and erythroid cells and comparing these with HeLa cells and lymphoblastoid cell lines. One of their interesting findings is that the Hox cluster is active in both NB4 cells and HeLa cells. The 3' end of the cluster is active in NB4 promyelocytic cells whereas the 5' end is active in HeLa cells. There is indication of a complex pattern of intergenic transcripts in this region and these investigators have been studying a transcript that is encoded between Hox A1 and Hox A2 and that is induced upon retinoic acid differentiation of the NB4 cells. Dr. Weissman pointed out that the advent of affordable massively parallel sequencing methodologies has transformative potential for genomic studies, and the Yale group is currently investigating areas of application where this approach may complement or replace microarray analysis.

Beyond transcriptional regulation of myelopoiesis: critical regulatory interactions

In previous workshops, reports of transcription factors influencing development of myeloid cells were common. However, as our knowledge has advanced the notion that these

transcription factors work within combinatorial networks or pathways and interact with other proteins has become well-accepted, and provided the basis for many talks at the present workshop. Interacting proteins may enhance binding by cooperation or may be involved in post-translational modifications that alter the transcription factor's activity.

According to Dr. A.D. Friedman (Johns Hopkins University, Baltimore, MD) interactions of C/EBP α and PU.1 in specifying myeloid lineages are more complex than previously thought. PU.1 is expressed before C/EBP α early in hematopoietic development. However, he proposes that later in development C/EBP α increases PU.1 levels to permit maturation of the common myeloid progenitor to the granulocyte-macrophage progenitor stage. Then, C/EBP α further induces PU.1 in cooperation with cytokine signals and additional transcription factors to promote monocyte development. He described, at the workshop and in a recent publication, new evidence demonstrating that C/EBP α directly induces PU.1 gene transcription¹⁷. Having previously found that C/EBP α induces endogenous PU.1 mRNA expression and binds and activates the PU.1 promoter, his group has now found that C/EBP α strongly binds and activates the PU.1 distal enhancer located at -14 kb. Using marrow cells from mice developed by Dr. D. Tenen that lack the distal enhancer, he also found that induction of PU.1 expression contributes to the ability of C/EBP α to direct monocyte as opposed to granulocyte lineage commitment *in vitro*. Dr. Friedman has also found that C/EBP α can directly zipper with c-Jun, JunB, or c-Fos to bind novel DNA elements, and so potentially activate novel genetic targets. Using acidic and basic leucine zippers to force specific heterodimerization in transduced marrow cells, Dr. Friedman's group found that C/EBP α :c-Jun or C/EBP α :c-Fos heterodimers induce monopoiesis more potently than C/EBP α :C/EBP α or c-Jun:c-Jun homodimers or c-Jun:c-Fos heterodimers. C/EBP α :c-Jun binds and activates the PU.1 promoter, potentially accounting for induction of monopoiesis. However, both G-CSF and M-CSF signals induce c-Jun phosphorylation to a similar extent, leaving open the question of how upstream signals cooperate with C/EBP α and c-Jun to direct myeloid lineage commitment.

Dr. A. Khanna-Gupta (Yale University, New Haven, CT) reported that a post-translational modification of C/EBP α , sumoylation, is associated with its negative regulatory activity during neutrophil development. Sumoylated C/EBP α levels decrease upon neutrophil maturation, and transactivation of the lactoferrin promoter (LF) reporter is enhanced by a sumoylation mutant of C/EBP α (K159A). Furthermore, evidence was provided that sumoylated C/EBP α binds to the C/EBP site in the LF promoter in uninduced myeloid cells while loss of sumoylation correlates with loss of binding. A shorter isoform of C/EBP α , CHOP, was shown to remove C/EBP α from binding to the LF promoter and to mediate a decline in the ability of C/EBP α to transactivate the promoter. Since levels of CHOP increase during neutrophil differentiation and CHOP itself does not recognize C/EBP *cis* elements, it is hypothesized that sumoylated C/EBP α binds the LF promoter in early myeloid cells and is associated with negative expression. Upon induction, levels of CHOP increase in the maturing neutrophil, inducing heterodimerization with presumably unsumoylated C/EBP α . This would now allow C/EBP α to bind, resulting in high level LF expression.

Sumoylation of C/EBP was reported by Dr. A. Leutz (Max Delbrueck Center for Molecular Medicine, Berlin, Germany) to be required for further modifications of the protein, including phosphorylation and arginine methylation. Mutational analysis revealed that phosphorylation, sumoylation, and arginine methylation occur in an interdependent fashion and abrogation of any of these modifications affects distinct biological functions of C/EBP, including proliferation and differentiation of myeloid cells and osteoblasts.

Two papers widened our understanding of the complexities of c-Myb function. c-Myb has been known as a transcription factor important for maintaining the immature state of myeloid cells and to be involved in proliferation. Dr. A.M. Gewirtz (University of Pennsylvania, Philadelphia, PA) presented exciting data that showed that this protein also regulates transcription by an alternative mechanism. C-Myb was shown to associate with components of the MLL-complex as well as the tumor suppressor protein menin, a product of the *MEN1* gene that is mutated in familial multiple endocrine neoplasia type 1. Importantly, c-Myb-containing complexes were able to methylate histone H3. These results suggest that c-Myb regulates transcription via another paradigm, that of covalent histone modification. In another talk on c-Myb, Dr. S. Ness (University of New Mexico Health Sciences Center, Albuquerque, NM) elaborated on his data that shows that the *c-myb* gene produces, through alternative RNA splicing, a number of transcripts that encode slightly different version of the c-Myb transcription factor. Transcription assays showed that the different versions of c-Myb had unique transcriptional activities and activated different genes when expressed in myeloid cells.

Dr. A.N. Goldfarb and coworkers (University of Virginia, Charlottesville, NC) have discovered a novel pathway of gene regulation by which a transcription factor's interacting partner recruits a kinase necessary for its transactivation function¹⁸. RUNX1 and GATA-1 both play essential roles in the transcriptional programming of normal mammalian megakaryocytic development. Physical and functional interactions between these two factors occur in mammals as well as in *Drosophila*. The Goldfarb lab mapped the conserved subdomain within the GATA-1 amino terminus that is both necessary and sufficient for transcriptional cooperation with RUNX1. This subdomain is not found in GATA-2, a family member which binds to RUNX1 but does not promote megakaryocytic maturation. Dr. Goldfarb reported that GATA-1 induces RUNX1 phosphorylation at recognition sites for cyclin-dependent kinases (cdks). Mutagenesis of RUNX1 further identified serine/threonine-proline S/TP sites collectively required for cooperation with GATA-1.

Results from Dr. P.G. Lutz's lab (CNRS, Toulouse, France) add to the growing evidence for the involvement of polyubiquitinylation and proteasomal degradation in control of hematopoiesis. They had previously identified *ASB2* (Ankyrin repeat-containing protein with a Suppressor of Cytokine signaling Box-2), a gene that encodes the specificity subunit of an E3 ubiquitin ligase complex protein involved in induced differentiation of myeloid leukemia cells¹⁹. The recent identification of ASB2 partners has revealed an unexpected link between ASB2 and cytoskeletal proteins. The stability of an actin-binding protein identified in this manner is regulated through ASB2 ubiquitin ligase activity.

Normal and leukemic stem cells (LSC)

Due to the recent focus on the stem cell properties of leukemic cells, the topic of stem cells at the workshop was indeed of high interest to participants. Leukemic stem cells (LSCs) are a small population of cells within the leukemic cell population which can transfer the disease to immunocompromised NOD/scid mice and have properties of normal stem cells including the ability to self-renew, multilineage differentiation, quiescent maintenance and drug resistance^{1,3}. Molecular studies on the hematopoietic microenvironment are much needed to improve our understanding of the maintenance of stem cells and their development. Therefore, it was refreshing to hear a talk by Dr. J.R. Keller (National Cancer Institute-Frederick, MD) describing his new findings for a role of the inhibitor of DNA-binding protein 1 (Id1) in the hematopoietic environment. Previously his lab had shown that Id1 is expressed at low levels in hematopoietic stem cells but is increased in more committed myeloid progenitor cells. Furthermore, overexpression of Id1 promotes myelopoiesis *in vivo*, suggesting that Id1 might regulate myeloid development²⁰. Id1^{-/-} mice, which were used to clarify the physiologic role of Id1, were reported to have a reduced myeloid compartment in the bone marrow and increased granulocytes in the peripheral blood. Transplantation of Id1^{+/+} bone marrow cells to Id1^{-/-} recipient mice reproduced the hematological phenotype, suggesting the defective bone marrow microenvironment was responsible for the observed abnormalities in Id1^{-/-} hematopoiesis. In correlation with these findings was the observation that expression of genes essential for bone marrow microenvironment was deregulated in Id1^{-/-} stromal cells and long-term culture-initiating cell (LTC-IC) assays showed increased progenitor proliferation on Id1^{-/-} stroma.

AML and chronic myeloid leukemia (CML) have been demonstrated to arise from clonal expansion of a single cell, and the disease can be sustained by a small population of LSC^{21,22}. LSC share many features of normal HSC, including quiescent properties, self-renewal potential and strong proliferative capacity. Acute promyelocytic leukemia (APL) which is associated with PML-RAR γ may be a subtype that is an exception to this primitive cell model for leukemia. Some evidence suggests it arises from cells that are more mature than stem cells, although it is still a controversial issue. Dr. S. Kogan (University of California, San Francisco, CA) is using his MRP8 PML-RARA mouse model to address this issue. He reported that most leukemic cells are CD117 (c-kit) and LY6 (Gr1) positive and such cells may have the capacity to transfer disease to recipient animals. Thus, data from his lab supports the idea that APL does not arise from the most primitive hematopoietic cells.

Dr. C. Eaves' laboratory (Terry Fox Laboratory, Vancouver, BC) has found that CML stem cells (SC) possess multiple unique properties that predict intrinsic and acquired resistance to *BCR-ABL*-targeted therapies²³. They recently demonstrated that highly enriched populations of CML SCs (lin-CD34+CD38-) are less responsive to imatinib (IM), express elevated levels of *BCR-ABL*, and have greater tyrosine kinase activity than the bulk of lin-CD34+CD38- leukemic cells. As reported by Dr. X. Jiang from the Terry Fox Laboratory (Vancouver, BC), they have obtained evidence that *BCR-ABL* expression decreases IM sensitivity. Interestingly, they also found that two transporter genes that regulate intracellular levels of IM are altered in CML. Expression of OCT1 (which regulates IM uptake) was found to be very low in the most primitive (lin-CD34+CD38-) of normal bone marrow

cells, and was increased (> 100-fold) in the most mature (lin+CD34-) progeny. This difference was markedly enhanced in CML samples. Conversely, transcript levels for ABCB1 (which regulates the efflux of IM) were highest in normal SC population and lowest in the most mature normal cells, and again this difference was enhanced in the corresponding leukemic cells.

Epigenetic mechanisms in leukemia

Classification of AML for prognostic purposes and therapeutic decision-making has had its limitations, since 40–50% of patients with AML present with a normal karyotype. In addition, the value of gene expression profiling hasn't reproducibly predicted AML subtypes. It is now realized that new oncogenes and tumors suppressors can be identified based upon epigenetic as well as genetic alterations. Epigenetic patterns are formed by methylation of DNA that can repress gene transcription as well as histone modifications that can alter gene expression patterns and chromatin structure²⁴. This is an area that deserves intense investigation if we are going to make advances in characterizing specific subsets of AML and finding new and improved therapeutic targets.

Dr. A.M. Melnick's laboratory (Albert Einstein College of Medicine, Bronx, NY) is dissecting molecular mechanisms underlying normal karyotype AML using an integrative genomic and epigenomic platform. Dr. M. Figueroa from his lab talked about their platform which consists of a combination of ChIP-chip, genome-wide DNA methylation using HELP, DNA copy number by arrayCGH, and gene expression arrays. They have shown that epigenetic platforms can be successfully used for leukemia classification and that each one of these platforms can capture a subset of unique genes not detected by the others. The use of epigenetic marks can help detect active genes that, although missed in the noise of the gene expression array, can be detected as having an active chromatin status, thus reflecting their availability for transcription. Some biological functions important in AML that were detected by epigenetic markers but not by gene expression include free-radical scavenging, inflammation and tumorigenesis, cell signaling, and energy production. Indeed a combination of genetic and epigenetic platforms can increase our ability to provide molecular classification of normal-karyotype AML.

Dr. N.J. Zeleznik-Le (Loyola University Chicago, Maywood, IL) informed attendees of the workshop that the mechanism by which MLL, a fusion partner found in leukemia, functions to maintain target gene expression is via epigenetic regulation. Mll was found to bind to specific clusters of CpG residues within the Hoxa9 locus, a well known target, and regulate expression of multiple transcripts, including one that encodes the canonical Hoxa9 protein. The presence of Mll provides protection from DNA methylation. This region also gives rise to Mll-dependent transcripts that can produce mir-196b microRNA. The microRNA expression increases with differentiation of murine ES and is dependent on the presence of Mll. Intriguingly, bone marrow progenitor cells expressing the leukemic MLL-AF9 have a greatly increased level of expression of mir-196b.

Myelodysplastic syndrome and myeloproliferative disease

Clonal disorders such as MDS and MPD affect even more individuals than overt leukemia, and because of this, the workshop organizers felt that research on these diseases should be included and expanded upon at future meetings. MDS refers to a group of stem cell disorders in which there are defects in hematopoiesis including erythrocytic, granulocytic and megakaryocytic lineages. Patients with these syndromes typically have hypercellular bone marrows, peripheral cytopenias, and cell functional abnormalities. The MPDs are a heterogeneous group of diseases that involve transformation of the HSC and include chronic myeloid leukemia (CML), polycythemia vera (PV) essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (IMF). With the exception of IMF, MPDs are characterized by increased production of blood cells in the absence of alterations in maturation. Many complications of MDS and MPD relate to overproduction of cells and/or cytopenias in the case of MDS. However, both can progress to leukemia. Recent data has demonstrated that myeloproliferative diseases, including PV, ET, IMF are all associated with activating mutations in the JAK2 tyrosine kinase. Dr. M. Carroll's laboratory (University of Pennsylvania, Philadelphia, PA) found that CEP701, known as an inhibitor of Flt3, is also an inhibitor of JAK2. Studies with primary patient material have demonstrated that CEP701 inhibits the growth of MPD samples, both those with and those without the JAK2 V617F mutation, without inducing apoptosis. The inhibitor decreased phosphorylation of multiple downstream signaling proteins, including STAT5 and Akt. Interestingly, CEP701 also rapidly decreased expression of the transcription factor nuclear factor erythroid 2(NF-E2), demonstrating that NF-E2, which is a known marker for PV, is a target of JAK2. Clinical trials in patients with MPDs are planned.

To determine the distinct role that JAK2V617F plays in the pathogenesis of PV, Dr. J Licht's laboratory (Northwestern University, Chicago, IL) reported experiments where they transduced human bone marrow CD34+ cells with either wild-type JAK2 or JAK2V617F. The mutant transfected cells showed a three-fold increase in erythroid differentiation in the presence of EPO compared to wild-type cells. To ascertain whether mutant JAK2 yielded an abnormal EPO response, Affymetrix array analysis was performed on WT and mutant transfected CD34+ cells with or without EPO. The results indicated that the abnormal EPO response in mutant transfected cells is more indicative of PV than the normal EPO response and that JAK2 mutation is a gain of function mutation at the genetic level, with a transcriptional readout exceeding that of normal EP signaling.

Using high resolution Affymetrix 250K Nsp SNP arrays, Dr. J. Licht's group sought to determine genomic copy number alterations that are associated with MPD disease phenotype. Amplification of 9p in a region harboring the JAK2 locus and 17q12.31 were the most common shared alterations in the MPDs while 20q deletion was only seen in ET patients. They, further, identified large alterations (>1Mb) found only in single patients suggesting sporadic genomic instability. The analysis also identified several recurrent large alterations.

Approximately 30%–50% of ET patients harbor an acquired valine 617 to phenylalanine point mutation in JAK2 (JAK2V617F). However, when expressed in a murine system, this

mutation leads to erythrocytosis or thrombocytosis but not to ET and the molecular etiology of this disease remains unknown. The fusion gene AML1/MDS1/EVI1 (AME), a product of the t(3;21)(q26;q22) translocation, is associated primarily with chronic myelogenous leukemia (CML) and myelodysplastic syndrome (MDS), and in one patient with ET. Dr. G. Nucifora (University of Illinois, Chicago) reported that a disease with symptoms and complications similar to human ET is reproducibly obtained in C57BL mice that express AME in their bone marrow. As observed in human ET, the animals did not have a unique phenotype but they all had abnormally high levels of dysfunctional platelets. At time of death, about half of the mice were severely anemic and cytopenic suggesting chronic bleeding while other animals had difficulty moving their limbs suggestive of hemiparesis. Finally, one of the mice developed acute myeloid leukemia, which is observed in about 10% of ET patients. Bone marrow of AME-positive mice showed normal erythropoiesis and granulopoiesis but were especially remarkable for large and giant megakaryocytes with a tendency for clustering near the bone trabeculae. These findings coupled with markedly increased levels of Tpo and PF4 but normal concentrations of c-Mpl are concordant with the morphologic and laboratory features of patients with ET. At time of death, the AME-positive mice did not harbor mutation of the Jak2 gene, indicating that this novel murine model could be valuable for better understanding the progression of the disease especially in the majority of ET patients who have wild-type JAK2 alleles.

According to Dr. L. Li from Dr. D. Small's laboratory (Johns Hopkins University, Baltimore, MD) a knock-in of an internal tandem deletion (ITD) of FLT3 into murine FLT3 confers myeloproliferative disease in a mouse model. Activation of FLT3 by internal tandem duplication in the juxtamembrane domain is the most common molecular alteration known in AML and confers poor prognosis. They generated the FLT3/ITD knock-in mouse model to study the in vivo biological impact of FLT3/ITD mutations in the development of leukemia. Young FLT3^{wt/ITD} show signs of MPD, which progress to fatality at the age of 6–20 months. Their data indicate that expression of a FLT3/ITD mutation alone is capable of partially transforming normal hematopoietic stem cells and progenitors to a phenotype of MPD. Additional cooperative events are likely required to progress to leukemia.

Recapitulation of human AML in mouse models

As in previous workshops, there were several talks on recapitulation of human AML in mouse models. Dr. J.C. Mulloy (University of Cincinnati College of Medicine, Cincinnati, OH) made considerable progress in modeling, in mice, disease induced by MLL-AF9, which is encoded by t(9;11)(p22;q23) in human AML. The MLL-AF9 translocation is associated with M5 monocytic leukemia and B cell acute lymphocytic leukemia (B-ALL). Previous mouse models involving this fusion protein have elegantly defined the nature of the leukemia stem cell implicated in AML-associated 11q23 leukemias²⁵. However, they have proven more limited in modeling the lymphoid and biphenotypic aspects of the disease. Dr. Mulloy reported that expression of MLL-AF9 in human CD34+ cells and transplantation into immunodeficient mice enables efficient modeling of mixed-lineage leukemia. The mixed-lineage nature of the leukemia stem cells was highlighted by the fact that the lineage of the resulting leukemia could be readily manipulated by altering either the growth factors or the recipient strain of mouse. Gene expression data from MLL-AF9-expressing cultured

cells revealed a transcriptional signature that closely mirrors that observed in MLL-rearranged leukemic cells from patient samples. This model promises to yield valuable insights into the molecular pathogenesis of MLL fusion-driven leukemia and will serve as a powerful in vivo model for testing therapeutic targets.

The power of insertional mutagenesis in murine models for AML was evident from the talk by Dr. C. Stocking (Heinrich-Pette-Institut, Hamburg, Germany). She and her colleagues have implicated a new gene *Mef2c* in transformation leading to AML, a gene that her group initially discovered applying insertional mutagenesis in *Icsbp*^{-/-} mice. The Mef2c of the MADS transcription factor family was first identified as important in regulation of muscle development in mammals. More recently their role in neuronal development has been revealed. Using a retroviral transduction/transplantation model, the Stocking lab confirmed the synergistic interaction of Icsbp deficiency and Mef2c over-expression in the induction of highly invasive, acute myelomonocytic leukemia. In vitro colony assays of bone marrow cells either with deficient or excessive levels of Mef2c showed that the Mef2c induces monocytic differentiation at the expense of granulopoiesis. The ability of Mef2c to induce c-Jun expression, which in turn interferes with C/EBP function, was demonstrated to be the likely mechanism. High levels of MEF2C transcripts were also observed in patient samples of myelomonocytic/monocytic leukemia, but preferentially in those with *MLL* gene disruptions.

Oncogene cooperation in AML

Because multiple genetic insults accumulate to cause AML, it is important to know which players cooperate with each other. In a couple of talks, genes that cooperate with oncogenic transgenes in mice were identified by retroviral insertional mutagenesis. For example, Dr. K. Keeshan and coworkers (University of Pennsylvania, Philadelphia, PA) identified Tribbles homolog2 and HoxA9 as cooperating genes in AML. This group had previously published that Trib2 inactivates C/EBP α and causes acute myelogenous leukemia in mice²⁶. Mice reconstituted with hematopoietic stem cells (HSC) uniformly developed a fatal transplantable disease. Since the AML's were clonal, Dr. Keeshan sought to identify by proviral insertion analysis candidate cooperating genes. She reported at the workshop that insertional mutagenesis of HoxA9 was discovered in a monoclonal tumor. Furthermore, when mice were reconstituted with HSC co-transduced with HoxA9 and Trib2, they had accelerated onset of AML compared to either gene alone.

Dr. T. Nakamura (Japanese Foundation for Cancer Research, Tokyo, Japan), in his studies on HoxA9/Meis1-induced myeloid leukemia, also identified another member of the Trib family of serine/threonine kinase-like proteins, Trib1, as a collaborator. Three of six common integration sites, Trib1, Evi1, and Ahi1, which were identified in their model, were up-regulated by retroviral integration and cooperation between Trib1 or Evi1 and HoxA9/Meis1 was reported to be exhibited both in vivo and in vitro.

Another example of gene cooperation in transformation of myeloid cells was presented by Dr. G.C. Grosveld (St. Jude Children's Research Hospital, Memphis, TN). The gene encoding the transcriptional coactivator MN1 is not only the target of the reciprocal

chromosomal translocation (12;22)(p13;q12) in some patients with AML, but it is also over-expressed in AML specified by inv(16). Dr. Grosveld's group discovered that mice receiving transplants of bone marrow over-expressing *MN1* rapidly developed myeloproliferative disease²⁷. Furthermore, forced co-expression of MN1 and Cbfb-SMMHC (expressed in inv(16) AML) rapidly caused AML in mice. These results suggest that MN1 over-expression is an obligatory and defining cooperative event in human inv(16) AML.

Children with Down syndrome (DS) display macrocytosis, thrombocytosis, and have a 500-fold increased risk of developing megakaryocytic leukemia. Although recently it was shown that acquired mutations in the megakaryocytic regulator GATA1 have been found in essentially all cases of DS, there could also be other specific effects of the trisomy 21 that impact hematopoiesis and AML²⁸. To investigate the impact of other genes, Dr. J. Crispino (Northwest University, Chicago, IL) has been using a mouse model for DS, the Ts65Dn mice. These mice are trisomic for 104 orthologs of Hsa21 genes and display persistent macrocytosis and develop MPD characterized by profound thrombocytosis, dysplastic megakaryocyte hyperplasia and myelofibrosis. Of the 104 trisomic genes in this strain, *Runx1*, according to Dr. Crispino, has been arguably the most promising leukemic predisposing gene. When his lab generated animals that are trisomic for 103 genes and disomic for *Runx1* they discovered that trisomy for Runx1 was not required for megakaryocyte hyperplasia and myelofibrosis. Of the remaining genes, ETS2 and ERG stand out as the most promising candidates for oncogenes since their over-expression in murine fetal liver progenitors resulted in a marked expansion of the megakaryocyte lineage, and ERG immortalized hematopoietic progenitors in vitro. These results suggest that increased expression of Ets family members may contribute to leukemic transformation in people with DS.

Mechanisms of transformation by oncogenic proteins

Aberrant fusion transcription factors encoded at translocation breakpoints represent a major portion of oncogenic proteins identified in AML. There were two papers reporting research on mechanisms by which AML1-ETO expressed at the t(8;21) translocation transforms myeloid progenitors²⁹. Dr. Y. Sauntharajah (University of Illinois at Chicago, Chicago, IL) described that introduction into cells of an abnormal RUNX1 (AML1) variant such as AML1-ETO results in down-regulation of JAK2 expression, and consequently, GM-CSF-mediated STAT5 phosphorylation. However, G-CSF continues to phosphorylate STAT5 through JAK3. The resulting decreased JAK repertoire in abnormal RUNX containing cells is a therapeutic opportunity, resulting in specific vulnerability of these cells to JAK3 inhibitors or GM-CSF. Dr. D-E. Zhang (The Scripps Research Institute, La Jolla, CA) proposed that AML1-ETO might have opposing effects on gene expression depending on the various conditions of the cellular environment. Besides repressing the MDR1 promoter in C33A and CV-1 cells, AML1-ETO strongly activates the promoter in K562 and B210 cells. Importantly, although this activation requires both the AML1 and ETO portions of the fusion protein, it did not depend on the AML1 binding site in the MDR1 promoter. This activation is likely through influence on the general transcriptional machinery rather than promoter-specific factors.

Dr. S. Hiebert's laboratory at Vanderbilt University (Nashville, TN) has been investigating another fusion partner of RUNX1, MTG16, in order to gather more information about the physiological functions of the MTG/ETO family. Using mice lacking *Mtg16* they found that the gene impairs the rapid expansion of stem/progenitor cells, which is required after bone marrow transplantation. Their investigation of the mechanistic basis of these defects indicates that *Mtg16* is required to suppress HSC mobilization and loss of *Mtg16* impairs progenitor cell cycle progression, which can be complemented by the exogenous expression of *c-Myc*.

More than 40 proteins have been identified as partners of MLL in acute leukemia. The partner proteins are categorized based on their subcellular localization into either the nucleus or cytoplasm. Recent studies have proposed that the mechanism of transformation of the cytoplasmic proteins is mediated by their oligomerization. Gephyrin, which is involved in synaptic anchoring of the glycine receptor and certain GABAA receptor subtypes, is a rare partner of MLL. Dr. M. J. Thirman (University of Chicago, Chicago, IL), who has been studying this fusion protein, reported that gephyrin has multiple dimerization domains and that a complex interaction of 3 domains of the protein are critical for immortalization activity in methylcellulose colony-forming assays.

A fusion protein found in acute megakaryoblastic leukemia (AMKL or AML FAB-M7) is RBM15-MKL, encoded by the t(1;22) translocation. In order to understand the mechanism by which the aberrant protein induces leukemia Dr. D. S. Krause and colleagues at Yale University School of Medicine, New Haven, CT) are first investigating the normal functions of the RBM15 (RNA-binding motif protein 15) and MKL1 (also known as MAL or BSAC) genes. RBM15 was found to be expressed at highest levels in hematopoietic stem cells and gradually decrease during myelopoiesis while MKL1 increases during megakaryocytopoiesis. Interestingly, both stimulate the Notch signaling pathway.

Insights into AML through high throughput platforms

Although genetic lesions have already been identified in cases of AML, for example, numerous translocations and point mutations, there are presumed to be large sets of unidentified somatic alterations in tumor genomes. Dr. T. J. Ley (Washington University School of Medicine, St. Louis, MO) presented an impressive talk on his Institute's studies to identify somatic changes in the tumor genomes. He and co-investigators are using prospectively banked samples from the bone marrow (tumor) and skin (germ-line) of patients with *de novo* AML. RNA and DNA from these samples have been used for array-based expression profiling studies, for array-based comparative genomic hybridization (CGH) studies on ultra-high-resolution arrays (1.5 million oligomers, average probe spacing ~2 Kb) and for high-resolution array-based SNP analysis (500K Affy SNP arrays). Using these strategies they have identified somatic mutations in several novel tyrosine kinase genes that are highly expressed in most AML samples, and they have identified somatic mutations in several other genes that have not previously been implicated in AML pathogenesis

Dr. R. Delwel's group (Erasmus MC, Rotterdam, The Netherlands) has recently been carrying out gene expression profiling of AML, and this has led to the identification of a

previously unrecognized subgroup of biphenotypic myeloid/T-cell acute leukemias. Dr. B. J. Wouters, from The Netherlands lab described this subgroup as one having an expression signature similar to that of AML that have C/EBPA mutants. In addition, this subgroup has frequent CEBA promoter hypermethylation and NOTCH1 mutations.

Dr. D. M. Loeb and Dr. M. A. McDevitt from Johns Hopkins University School of Medicine (Baltimore, MD) are collaborating on a systematic approach to evaluate tumor-specific alternative RNA splicing. They are doing this with the anticipation that they will be able to gain insight into pathways that might be targets of small molecule inhibitors and to develop new diagnostic and prognostic tools. They have designed a 44K leukemia-specific custom splice array for this purpose. Already, they have used the array to identify and confirm 45 genes in CML that have alternative splicing in multiple cells lines. Among these are genes such as the axl tyrosine kinase which has previously been associated with CML.

Conclusions

State-of-the-art research on myelopoiesis and leukemia, as reported at the Seventh International Workshop on Molecular Aspects of Myeloid Stem Cell Development and Leukemia, continues to make significant progress in defining the molecular mechanisms that regulate stem cell biology, normal hematopoiesis and the leukemic transformation. Knowledge of the transcriptional regulation of lineage specification continues to expand, with novel insights into how transcriptional promiscuity in stem cells and early progenitors is resolved during differentiation, and how post-transcriptional and epigenetic controls participate in regulating these processes. The biology of normal and leukemic stem cells, and the mechanisms that regulate myelodysplastic syndromes and myeloproliferative disorders continue to receive considerable attention and yield novel insights. Both mouse models that recapitulate human myelodysplastic syndromes and leukemias, and genomic screens for novel transcripts that regulate normal and dysplastic myelopoiesis, contribute to our overall understanding of the cellular and molecular pathways that drive normal stem cell development and abnormal hematopoiesis. Reports of novel therapeutic targets for leukemia suggest that we have only just begun to uncover approaches to selectively inhibit critical oncogenic pathways in the hematopoietic system.

Acknowledgments

The Seventh International Workshop on Molecular Aspects of Myeloid Stem Cell Development and Leukemia was generously sponsored by the Center for Cancer Research (CCR) of the National Cancer Institute (NCI, NIH) (to LW), with extramural support provided by R13 conference grant HL088917 (to SJA) from the National Heart Lung and Blood Institute (NHLBI/NIH) (co-funded by the NCI), and a generous grant (to GN) from the Leukemia and Lymphoma Society (LLS). Additional support was generously provided by Ortho Biotech Partners, LP (to GN).

References

1. Buzzeo MP, Scott EW, Cogle CR. The hunt for cancer-initiating cells: a history stemming from leukemia. *Leukemia*. 2007; 21:1619–1627. [PubMed: 17541397]
2. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*. 1997; 3:730–737. [PubMed: 9212098]
3. Dick JE, Lapidot T. Biology of normal and acute myeloid leukemia stem cells. *Int J Hematol*. 2005; 82:389–396. [PubMed: 16533740]

4. Laiosa CV, Stadtfeld M, Graf T. Determinants of lymphoid-myeloid lineage diversification. *Annu Rev Immunol.* 2006; 24:705–38. 705–738. [PubMed: 16551264]
5. de Jong JL, Zon LI. Use of the zebrafish system to study primitive and definitive hematopoiesis. *Annu Rev Genet.* 2005; 39:481–501. [PubMed: 16285869]
6. Lyons SE, Lawson ND, Lei L, et al. A nonsense mutation in zebrafish *gata1* causes the bloodless phenotype in vlad tepes. *Proc Natl Acad Sci U S A.* 2002; 99:5454–5459. [PubMed: 11960002]
7. Koschmieder S, Rosenbauer F, Steidl U, Owens BM, Tenen DG. Role of transcription factors *C/EBPalpha* and *PU.1* in normal hematopoiesis and leukemia. *Int J Hematol.* 2005; 81:368–377. [PubMed: 16158816]
8. DeKoter RP, Kamath MB, Houston IB. Analysis of concentration-dependent functions of *PU.1* in hematopoiesis using mouse models. *Blood Cells Mol Dis.* 2007
9. DeKoter RP, Singh H. Regulation of B lymphocyte and macrophage development by graded expression of *PU.1*. *Science.* 2000; 288:1439–1441. [PubMed: 10827957]
10. Dahl R, Walsh JC, Lancki D, et al. Regulation of macrophage and neutrophil cell fates by the *PU.1:C/EBPalpha* ratio and granulocyte colony-stimulating factor. *Nat Immunol.* 2003; 4:1029–1036. [PubMed: 12958595]
11. Laslo P, Spooner CJ, Warmflash A, et al. Multilineage transcriptional priming and determination of alternate hematopoietic cell fates. *Cell.* 2006; 126:755–766. [PubMed: 16923394]
12. Dahl R, Iyer SR, Owens KS, Cuyler DD, Simon MC. The transcriptional repressor *GFI-1* antagonizes *PU.1* activity through protein-protein interaction. *J Biol Chem.* 2007; 282:6473–6483. [PubMed: 17197705]
13. Du J, Stankiewicz MJ, Liu Y, et al. Novel combinatorial interactions of *GATA-1*, *PU.1*, and *C/EBPepsilon* isoforms regulate transcription of the gene encoding eosinophil granule major basic protein. *J Biol Chem.* 2002; 277:43481–43494. [PubMed: 12202480]
14. Rosu-Myles M, Taylor BJ, Wolff L. Loss of the tumor suppressor *p15Ink4b* enhances myeloid progenitor formation from common myeloid progenitors. *Exp Hematol.* 2007; 35:394–406. [PubMed: 17309820]
15. Resendes KK, Rosmarin AG. *GA-binding protein* and *p300* are essential components of a retinoic acid-induced enhanceosome in myeloid cells. *Mol Cell Biol.* 2006; 26:3060–3070. [PubMed: 16581781]
16. Yang ZF, Mott S, Rosmarin AG. The Ets transcription factor *GABP* is required for cell-cycle progression. *Nat Cell Biol.* 2007; 9:339–346. [PubMed: 17277770]
17. Yeaman C, Wang D, Paz-Priel I, et al. *C/EBP{alpha}* binds and activates the *PU.1* distal enhancer to induce monocyte lineage commitment. *Blood.* 2007
18. Elagib KE, Goldfarb AN. Regulation of *RUNX1* transcriptional function by *GATA-1*. *Crit Rev Eukaryot Gene Expr.* 2007; 17:271–280. [PubMed: 17725493]
19. Heuze ML, Guibal FC, Banks CA, et al. *ASB2* is an *Elongin BC*-interacting protein that can assemble with *Cullin 5* and *Rbx1* to reconstitute an *E3 ubiquitin ligase complex*. *J Biol Chem.* 2005; 280:5468–5474. [PubMed: 15590664]
20. Leeanansaksiri W, Wang H, Gooya JM, et al. *IL-3* induces inhibitor of DNA-binding protein-1 in hemopoietic progenitor cells and promotes myeloid cell development. *J Immunol.* 2005; 174:7014–7021. [PubMed: 15905544]
21. Holyoake TL, Jiang X, Drummond MW, Eaves AC, Eaves CJ. Elucidating critical mechanisms of deregulated stem cell turnover in the chronic phase of chronic myeloid leukemia. *Leukemia.* 2002; 16:549–558. [PubMed: 11960331]
22. Warner JK, Wang JC, Hope KJ, Jin L, Dick JE. Concepts of human leukemic development. *Oncogene.* 2004; 23:7164–7177. [PubMed: 15378077]
23. Jiang X, Zhao Y, Smith C, et al. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. *Leukemia.* 2007; 21:926–935. [PubMed: 17330101]
24. Esteller M. Epigenetics provides a new generation of oncogenes and tumour-suppressor genes. *Br J Cancer.* 2006; 94:179–183. [PubMed: 16404435]
25. Somervaille TC, Cleary ML. Identification and characterization of leukemia stem cells in murine *MLL-AF9* acute myeloid leukemia. *Cancer Cell.* 2006; 10:257–268. [PubMed: 17045204]

26. Keeshan K, He Y, Wouters BJ, et al. Tribbles homolog 2 inactivates C/EBPalpha and causes acute myelogenous leukemia. *Cancer Cell*. 2006; 10:401–411. [PubMed: 17097562]
27. Grosveld GC. MN1, a novel player in human AML. *Blood Cells Mol Dis*. 2007; 39:336–339. [PubMed: 17698380]
28. Muntean AG, Ge Y, Taub JW, Crispino JD. Transcription factor GATA-1 and Down syndrome leukemogenesis. *Leuk Lymphoma*. 2006; 47:986–997. [PubMed: 16840187]
29. Mikhail FM, Sinha KK, Sauntharajah Y, Nucifora G. Normal and transforming functions of RUNX1: a perspective. *J Cell Physiol*. 2006; 207:582–593. [PubMed: 16250015]

Figure 1.

Development of myeloid cells from the hematopoietic stem cell. The transcription factors that are lineage-instructive are shown next to each progenitor; this is based upon ref ⁴. HSC, hematopoietic stem cell; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte-erythroid progenitor; GMP, granulocyte-macrophage progenitor.