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Title:

Insights into cell ontogeny, age and acute myeloid leukaemia

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Abstract

Acute Myeloid Leukaemia (AML) is a heterogenous disease of haematopoietic stem and progenitor cells (HSCs/HSPCs). The pathogenesis of AML involves cytogenetic abnormalities, genetic mutations and epigenetic anomalies. Whilst it is widely accepted that the cellular biology, gene expression and epigenetic landscape of normal HSCs changes with age, little is known about the interplay between the age at which the cell becomes leukaemic and the resultant leukaemia. Despite its rarity, childhood AML is a leading cause of childhood cancer mortality. Treatment is in general extrapolated from adult AML on the assumption that adult and paediatric AML are similar biological entities. However, distinct biological processes and epigenetic modifications in paediatric and adult AML may mean that response to novel therapies may be different in children compared to adults with AML. A better understanding of the key pathways involved in transformation and how these differ between childhood and adult AML is an important step in identifying effective treatment. This review aims to highlight both the commonalities and differences between paediatric and adult AML disease biology with respect to age.

Acute myeloid leukaemia (AML) is a heterogeneous disorder characterised by an uncontrolled proliferation and differentiation block in immature hematopoietic stem and progenitor cells (HSPC) resulting in an accumulation of immature blasts. AML is rare in children occurring at an incidence of 8/million/yr in children 1-18 years of age. In adults the incidence rate of AML further increases, reaching 20/million/yr in persons between the ages 18-60 years and 170/million/yr in persons over the age of 60. Considering this age-related profile, it is reasonable to assume AML would develop at the lowest incidence in infants. However, infants have the highest incidence of AML within the paediatric population occurring at a rate of 15/million/yr in infants under the age of 1[1]. Distinct genetic abnormalities occur in infants, and together with the higher incidence rate, strongly suggest that infant AML is a separate biological entity. Age remains an independent predictor of outcome with superior survival associated with younger age[2, 3]. Overall survival (OS) is lower with increasing age; persons between 0-18 years of age have an OS of 70-75% versus 45-50% for persons between the ages of 18-60 years[4-8]. Increased OS in children is associated with a lower relapse rate (RR) reflecting a more chemo-responsive disease and less co-morbidity thus better tolerance of treatment. Age is one of a number of important independent prognostic factors; others include presenting white cell count, cytogenetic/molecular abnormalities, antecedent myelodysplasia and early response to treatment. The appropriateness of the current practice of extrapolating treatments across the age spectrum is dependent on the assumption that the same aetiology underlies AML in the young and old. However, differences in disease characteristics between paediatric and adult AML have been reported[3, 9], and which will be considered in this review. A better understanding of age-dependent biological differences may lead to improvements in treatment for children. The World Health Organisation (WHO) classification in 2008 based upon underlying cytogenetic or molecular genetic abnormalities, applies to both adult and paediatric AML[10]. The clinical differences between adults and children have been recently reviewed[11] and key differences are summarised in Table 1. Incidence and prognosis are clearly different, as well as differences in the prevalence of genetic anomalies and prognostic implications, which will be discussed further. While one might expect that paediatric and adult AML are completely different diseases, in fact there is a spectrum of similarities and differences between the two, and a better understanding of these will help guide management in both childhood and adult disease.

Age and genetic abnormalities in AML

It is clear that the genetic profile of childhood and adult AML is vastly different (Table 1). Childhood AML is characterised by cytogenetic abnormalities while adult AML is characterised by cytogenetically normal (CN) disease with a high proportion of molecular defects. The prognostic importance of cytogenetic abnormalities is well-established in the patient risk stratification of good, intermediate and poor risk groups. The cytogenetic abnormalities of the core binding factor (CBF) leukaemias, $t(8;21)(RUNX1/RUNX1T1)$ and $inv(16)(CBFB/MYH11)$, and $t(15;17)(PML/RARA)$ classifying acute promyelocytic leukaemia (APML; FAB classification M3), have similar morphology, gene expression and response to treatment in paediatric and adult AML and carry the same favourable prognosis in children as in adults[2, 12]. Good risk is more prevalent in paediatric compared to adult AML. Within paediatric AML, good risk disease is more common in children between 1 to 10 years of age compared to adolescents, which contributes to the superior outcome observed in paediatric AML. Currently there is a gap in our knowledge whether other genetic anomalies occurring in an adult, such as monosomy 7 which confers poor risk, have the same poor prognostic implications in a child. Stem cell transplantation (SCT) in 1st complete remission (CR) is recommended in adults with standard or high risk disease as it may result in superior disease free survival (DFS) and OS[13]. Poor risk cytogenetics in adults include monosomy 5 or 7, 5q abnormalities as well as $inv(3)$, $t(3;3)$, $abn(3q)(EVI1)$, $t(6;9)(DEK/NUP214)$, $t(9;22)(BCR/ABL)$ and complex cytogenetics (defined as ≥ 3 , 4 or 5 unrelated abnormalities)[2, 14, 15]. While these same poor risk cytogenetic abnormalities identify a group of children who have a poorer response than those with a normal karyotype, the outcome is not always sufficiently poor in children to justify SCT in 1st CR[16]. In particular, complex cytogenetics confers very poor risk in adults[2, 17], however it is associated with inferior outcome only in some paediatric groups; in the absence of other poor risk features, AML in children with complex cytogenetics does not necessarily warrant SCT in 1st CR[12, 18]. This discrepancy in clinical outcome with age in genetically matched patients may be due to better tolerance of intensive chemotherapy at younger age, or due to different biological consequences resulting from the same genetic aberrations in paediatric and adult patients. Further molecular and cellular studies comparing genetically matched AML samples from children and adults needs to be undertaken in order to elucidate this point. It is important to consider genetic abnormalities that are more prevalent in childhood AML. 11q23 chromosome abnormalities of the

mixed lineage leukaemia (MLL) gene occur at a higher incidence in children compared to adults (38% versus 2% respectively) with the highest incidence in infants (77%). In contrast to adults in whom 11q23 abnormalities confer poor risk (excluding t(9,11) which denotes intermediate risk), only certain MLL translocations confer poor risk in children (table 1), while t(1,11) may confer good risk[19]. Poor risk genetic aberrations that occur almost exclusively in a paediatric population include the cryptic translocation t(5;11)(NUP98/NSD1)[20] as well as the novel inv(16)(CBFA2T3/GLIS2)[21] seen in non-Down Syndrome(DS)-acute megakaryoblastic leukaemia (AMKL; FAB classification M7), and t(7;12)(MNX1/ETV6)[22]. Both inv(16)(CBFA2T3/GLIS2 and t(7;12)(MNX1/ETV6) occur almost exclusively in infants.

The intermediate risk group across the age spectrum consists of patients with CN disease and cytogenetic aberrations not classed as good or poor. CN disease accounts for 50% of adult disease[2] but only 25% of childhood AML[12, 18]. In adults, molecular abnormalities further risk-stratify the heterogenous CN group[14, 15]. CN prognostic implications are similar in adult and paediatric AMLs suggesting similar downstream pathways from these molecular abnormalities. However, they are present at a far less frequency in paediatric AML. For example, internal tandem duplicates of the FMS-like tyrosine kinase 3 (FLT3ITD) are present in 10% of children but at a much higher frequency of 20-40% in adults with CN AML and both are associated with increased RR[23-25]. Mutations of nucleophosmin (NPM1) do not occur in infants, but occur in 5-10% of children and much higher incidence of 35% of adults and confer good risk in the absence of a FLT3ITD mutation[25-28]. Biallelic mutations of the CCAAT enhancer-binding protein alpha (CEBPA) occur in 5% of childhood AML and 10% of adult AML and confer good risk in CN patients with improved RR, again in patients without a FLT3ITD mutation[29-31]. Mutations in the epigenetic modifiers DNMT3A (DNA methyl transferase 3A) and TET2 (ten-eleven-translocation methylcytosine dioxygenase 2) confer poor risk in adult AML but are rarely found in paediatric AML[32-35]. Recent studies have identified these two mutations in particular to be early events in adult AML, resulting in the production of a preleukaemic clone that predates clinical AML and evades therapy. The very low incidence of these mutations in childhood AML strongly suggests that the pathogenic process of AML development and cause of relapse is very different in childhood. Collectively, the stark differences between the genetic abnormalities occurring in childhood and adult AML (some occurring exclusively in children) suggest a different biological mechanism contributes to the pathogenesis of AML arising at different

ages. More insight into such age-specific biological mechanisms is warranted which would have clear and significant impact on precision driven therapies in the young and old.

Infant AML

Children less than 1 year of age presenting with AML form a distinct clinical and biological group. They fall into either myeloid leukaemia of Down Syndrome (ML-DS) or non DS-AML. In both ML-DS and non-DS AML there is evidence of a founding somatic mutation acquired *in utero* giving rise to a pre-leukaemic clone[36-39]. 5-10% of newborns with DS (trisomy 21) develop transient abnormal myelopoiesis (TAM) in the first 3 months of life[40], presenting as increased leucocytes, thrombocytopenia, circulating blasts and occasionally end organ failure due to accumulation of blasts[41]. This disorder is characterised by mutations in the GATA1 gene, which results in a truncated form of the protein[42]. This somatic mutation is acquired *in utero*, as evidenced by presence of GATA1 mutations in 4% of newborn blood spots from 585 DS newborns[38] and development of TAM/ML-DS with GATA1 mutation prenatally resulting in hydrops fetalis[39]. A high percentage of TAMs regress spontaneously without treatment. However, 19% of these patients will develop ML-DS of AMKL subtype in the subsequent 2-4 years[43-45]. There is a GATA1 mutation in all cases of ML-DS and interestingly, paired samples from patients with TAM who later developed ML-DS contained the same GATA1 mutation[46]. Thus, GATA1 mutations arising *in utero* results in a pre-leukaemic clone that can progress to ML-DS associated with further genetic insults. This strongly suggests that this pre-leukaemic clone is a distinct fetal entity, as ML-DS arising after 4 years of age is rare, and that in the absence of progression to AML this fetal derived pre-leukaemic clone naturally regressed after time. An important unanswered question is whether such a disease specific clone does not exist or occur in adults because of the age of the cell in which the mutations occurred, or because of inherent biological mechanisms in adult cells that prevents the survival of cells with such mutations though to adulthood.

Infants with non-DS AML present with myelomonocytic or monoblastic AML (M4 or M5)[47] and tend to have higher white cell counts than older children and often CNS involvement[48]. As well as the predominance of MLL rearrangements, rare aberrations almost exclusively seen in this cohort include $t(7;12)(MNX1/ETV6)$,[22] $t(1;22)(RBM15(OTT)-MKL1(MAL))$ [49] and $inv(16)(CBFA2T3/GLIS2)$ [21], the latter two associated with non-DS AMKL. In contrast, CBF AML and

APML are rare in infants[9, 50]. Like GATA1 mutations in ML-DS, MLL rearrangements occur *in utero* evidenced by twin concordance studies[36] and by the detection of MLL rearrangements in patient matched leukaemic samples and newborn blood spots [37]. It is strongly suggested that AML developed in the early postnatal period is due to the acquisition of a second genetic hit. Thus, infant AML most likely results from an initiating leukaemic event in a fetal cell. What has not been adequately addressed to date is whether MLL rearrangement present in a fetal cell leading to infant AML is the same disease as MLL rearrangement present in a young or older adult cell leading to childhood or adult AML.

Preclinical evidence linking ontogeny with disease phenotype

It is increasingly evident that cell intrinsic factors play an important role in determining phenotype following a genetic insult. Studies investigating the leukaemia initiating cell in several AML mouse models have shown that the cell type, be it a haematopoietic stem cell (HSC) or committed progenitor, has implications on cell transformation and leukaemic phenotype[51, 52]. Therefore, the same oncogenic abnormality can result in diverse disease biology. However, limited data is available on cell ontogeny and leukaemic transformation.

MLL rearrangements are associated with both AML and acute lymphoblastic leukaemia (ALL). The most common MLL rearrangement in adults and children over the age of 1 year is t(9,11) resulting in the fusion gene *MLL-AF9* and accounts for 22% of MLL rearrangements in infant AML, second only to t(10;11)(*MLLT10-AF10*)[53, 54]. Investigations into the effect of cell ontogeny on immortalisation, lineage commitment and gene expression have shown that following transduction of MLL-AF9 in human CD34^{+ve} cord blood (CB) and adult bone marrow (ABM) cells, both populations grew *in vitro*, however ABM gave rise to a myeloid bias. While both MLL-AF9 transduced CB and ABM cells were able to reconstitute the BM in a mouse transplantation setting, MLL-AF9 transduced ABM cells displayed long term engraftment with a myeloid predominance, without progression to leukaemia. In contrast, MLL-AF9 transduced CB cells consistently led to acute leukaemia. In the majority of cases acute lymphoblastic leukaemia developed, but one leukaemia shows mixed myeloid and lymphoid lineage. Transformed CB cells also retained a gene expression signature similar to normal neonatal HSCs[55]. While CB is not an exact surrogate for paediatric BM, this study provides and supports the conceptual basis that AML arising in a young and old cell may be very different.

Despite the same genetic aberration, age-dependent characteristics of the cell can profoundly influence self-renewal, transformation, and gene expression profiles, as evidenced by that study. In fact, gene expression differences related to stem cell age are retained in the leukemic cell. The implications from this study on therapy have not been further investigated.

Similar studies addressing cell ontogeny and the resulting leukaemia for the plethora of genetic aberrations that occur in AML are limited. The genetic aberration, Pten deletion, is seen in adult AML but rarely in paediatric AML. In normal haematopoiesis Pten maintains HSC quiescence by suppression of the PI3 kinase pathway, which promotes proliferation and cell cycling. In a murine model, deletion of Pten in adult HSCs (8 weeks old) led to activation of the PI3 kinase pathway and resulted in HSC proliferation, HSC depletion and resulted in leukaemia of either the myeloid or lymphoid lineage. Specifically, Pten deletion in young mice led to either a myeloproliferative disorder or T cell-ALL (T-ALL). Significantly however, Pten deletion in fetal and neonatal HSCs (14 days old), did not lead to PI3 kinase pathway activation nor result in leukaemia. Thus, tumour suppression programs that are critical in adults may be unnecessary in fetal or neonatal HSCs, resulting in differences in leukaemic susceptibility in young and old HSCs[56]. The highlighted studies on Pten and MLL-AF9 provide direct evidence that leukaemia initiation, development and phenotype are influenced strongly by the age of the cell. There remains a research void however in the therapeutic implication of such studies. Research into the cell of origin, the age and phenotype, using appropriate mouse models could help to address this open question.

Normal biology of haematopoietic cell ageing

The normal biology of aging in the murine and human haematopoietic system has been extensively reviewed[57] but the implications on AML to date, have not. Here we will highlight the key differences with aging in both systems and how they may impact on AML. The proliferative and differentiative potential of normal murine and human HSCs changes with age. In the mouse model there are differences in proliferation, lineage output and gene expression in HSCs from prenatal and neonatal (fetal to 3w of age), adult (>6-8w old) and aged (greater than 24 months) mice. Haematopoietic cells isolated from fetal liver (FL) are more efficient at repopulating the BM of lethally irradiated mice compared to cells from adult mice which assume a more quiescent phenotype[58, 59]. The progeny of transplanted FL cells have a myeloid preponderance while ABM produces a balanced

lineage output. As mice age, the absolute and relative numbers of HSCs increase but function diminishes, with loss of lymphoid potential, increased self renewal and accumulation of DNA damage[60-62]. This coincides with a change in gene expression profiles. Analysis of the transcriptional program of definitive HSC ontogeny in the normal setting showed that FL derived HSCs have higher expression of genes involved in cell cycling and self renewal while in contrast, adult derived HSCs have higher expression of genes involved in HSC maintenance[59, 63]. Comprehensive integrated genomic analysis of HSCs from 4 month and 24 month old mice has shown changes in the transcriptome, DNA methylation and histone modification that play a part in increasing DNA instability with increasing age[64]. Genes involved in cell adhesion, proliferation and protein synthesis are upregulated in aged HSCs while there is a downregulation of genes related to DNA replication, DNA repair and cell cycle[64]. Recently, replication stress was identified as a potent driver of functional decline in old HSCs linked with decreased mini-chromosome maintenance (MCM) gene expression in old HSCs, rather than the failure of HSCs to activate the DNA damage response[65]. Interestingly, recent work has shown that DNA damage response proteins are activated in response to oncogene expression and this response is actually required for tumourigenic function in contrast to the conventional view that the DNA damage response is anti-tumourigenic[66]. Thus, gene expression studies provide important information regarding the genetic profile that influences the ability of the cell to respond to stress, and possibly the propensity of the cell to be transformed by oncogene expression or tumour suppressor deletion.

Similar age related differences in human HSCs (defined as cells expressing the surface antigen CD34) have also been described in which fetal derived HSCs from fetal liver and cord blood had enhanced repopulating and differentiative potential when compared to HSCs derived from adult bone marrow[67]. When comparing young (20-35 years) and elderly (>65 years) adult CD34^{+ve} cells, the older cells resulted in a lower level of engraftment when transplanted into immunodeficient mice, and reduced B-lymphoid differentiation both *in vitro* and *in vivo*, while young adult CD34^{+ve} cells had balanced myeloid-lymphoid differentiation. This corresponds to a change in the gene expression profile, with up-regulation of genes involved in myeloid lineage determination, DNA repair and cell death in older adult CD34^{+ve} cells, as well as up-regulation in known leukaemia associated genes[68]. Measurement of telomere shortening as a surrogate for prior cell divisions suggest that HSCs in children divide rapidly in the first year, with an abrupt decline between 1-3 years and further decline

after 13 years to adult rates of cell division[69, 70]. Similar to murine models, aged human HSCs exhibit increased DNA damage, with an increase in double strand breaks and a diminished capacity for DNA repair[71]. This is supported by whole genome sequencing (WGS) studies of adult AML and healthy adults that reveal that HSCs accumulate somatic mutations with age[72]. Together our knowledge of normal stem cell biology highlights the age related differences in HSC proliferation, HSC lineage potential and HSC DNA stability that may have implications in age-related susceptibilities to AML (Figure 1).

Age and gene expression in AML

In the past two decades gene expression profiling has been undertaken to more accurately risk stratify AML, aiming to identify AML risk categories superior to those conventionally defined by clinical, cytogenetic and molecular methods. Gene expression profiling of AML has been shown to successfully predict cytogenetic risk categories[73, 74]. In particular, t(15;17), t(8;21) and inv(16) have distinctive gene expression signatures with high predictive value in validation sets. Gene expression profiling has also identified subclasses within CN patients[73, 74]. These subtypes have prognostic significance and may help to further risk stratify the heterogeneous CN group. These have been conducted in adult or paediatric sample sets. Data from paediatric patients are often included in predominantly adult datasets due to limitations in sample number. Critically, studies directly comparing paediatric and adult gene expression data are rare. One study of an adult cohort (525 patients) identified a specific gene expression profile which was associated with an inferior CR rate, event free survival and OS, independent of cytogenetic risk group. This specific profile also correlated with an inferior OS in a smaller paediatric set (100 patients)[75] indicating paediatric data is in line with the adult results for this profile. However, more studies like this need to be conducted so that the similarities and differences in gene expression, pathway activation and biological processes between paediatric and adult AML can be identified. In an exclusively paediatric cohort (n=130) gene expression profiling study distinct clusters were identified including t(15;17), t(8;21), inv(16), MLL chimeric genes and FAB M7 that could be used with 93% accuracy to predict clusters in both a validated paediatric and adult cohort[76]. Improved predictions with 99% accuracy could be made from gene expression signatures from one paediatric cohort (n=237) on exclusively paediatric validation sets[77]. This higher accuracy may reflect age related differences to be discovered when

comparing adult and paediatric datasets. We must also consider the response of young or old AML to treatment; gene expression studies comparing paediatric and adult cohorts in response to treatments may provide us with important insights into the biology of AML across the ages.

Clonal haematopoiesis and pre-leukaemia

Advances in cancer genomics have revealed the spectrum of somatic mutations that give rise to human AML and have drawn our attention to the molecular evolution and clonal architecture of AML. Recently, several publications have shown that normal haematopoietic cells harbouring known leukaemia driver mutations gain a competitive survival advantage and clonally expand with increasing age[78-80]. These studies suggest that clonal haematopoiesis arises from the expansion of pre-leukaemic cells expressing a driver mutation and may be an aspect of a normal ageing in the hematopoietic system. Whole-exome sequencing (WES) data from the peripheral blood of persons unselected for cancer or haematological disease revealed clonal haematopoiesis occurs in 10% of persons over 65 years of age, occurring in only 1% in <50 years, and found increased frequency of somatic mutations with increasing age in clonally expanded cells[78, 79]. Somatic gene mutations most frequently occurred in the epigenetic regulators DNMT3A, TET2 and ASXL1 (additional sex combs-like transcriptional regulator 1). These mutations predict age-related clonal haematopoiesis as a strong risk factor for the subsequent development of haematological malignancy. This is supported by previous work that implicates DNMT3A and TET2 mutations as early events that lead to the development of MDS and AML[72, 80, 81]. Other studies support the notion of a pre-leukaemic phenotype, whereby ancestral pre-leukaemic HSCs are able to regenerate the entire haematopoietic hierarchy while possessing competitive repopulation advantage over non-leukaemic HSCs, leading to clonal expansion; these pre-leukaemic cells can also carry DNMT3A, TET2 and ASXL1 mutations and are resistant to treatment compared to overt AML[82-85]. Collectively, these studies predict that mutations occurring in epigenetic regulators can occur in healthy adults and pre-date AML diagnosis by months or years, with secondary mutations in proliferative genes, such as NPM1 or FLT3-ITD translocations occurring at a later stage[83].

In adult AML, DNMT3A mutations are considered to be independently associated with unfavourable prognosis and positively correlate with increasing age[86]. DNMT3A mutations occurring

in 15-25% of adult AML are enriched in CN and intermediate risk subgroups and commonly co-occur with FLT3, NPM1 and IDH1/2 (isocitrate dehydrogenase 1/2) mutations[32, 87, 88]. IDH1/2 mutations occur in 15-30% of adult AML[89]. In contrast to adult AML, mutations in DNMT3A (1%) and IDH1/2 (0.9% and 0.48% respectively) are rarely observed in paediatric AML[90, 91]. In addition, mutations in the epigenetic regulators ASXL1 and TET2 have also been associated with an unfavourable prognosis in adult AML. TET2 mutations are found in 2-23% of adult AML in contrast to only 1.7% paediatric AML[91]. TET2 mutations in adult AML commonly co-occur with other somatic mutations including NPM1, RAR α , KIT, FLT3, RAS, MLL and C/EBP α [92-96]. ASXL1 mutations occur in adult AML (5-30%) more often in patients over 60 years (16%) than in patients 60 years and younger (3.2%). Unlike TET2, ASXL1 mutations are rarely found in conjunction with NPM1 or FLT3 mutations, but are associated with C/EBP α mutations[97, 98]. Interestingly, in a limited gene set, paediatric AML had fewer gene mutations than adult AML. While one cannot conclude that more mutations are required for the development of adult AML, it does highlight that the mutational background of paediatric and adult AML is different[91]. Data supports that in contrast to adult AML where mutations of epigenetic regulators commonly co-occur, epigenetic mutations occurring in paediatric patients, whilst rare, appeared to be mutually exclusive and occur with other non-epigenetic related gene mutations. In fact, paediatric cancers on average contain fewer somatic mutations than comparable tumours arising in adults[99]. We do not yet have any evidence for the occurrence of mutations in epigenetic regulators *in utero*; whether this is because studies have not investigated this or because such mutations do not exist at that stage is not currently known. Research to date suggests that mutations in epigenetic regulators are somatically acquired and leukaemia-associated in children with AML[91].

To date, it is clear that mutations in epigenetic modifiers are enriched in adult AML (16 years and above) and that the frequency of mutations in epigenetic modifiers correlates with the increasing age of AML patients[97, 98, 100]. The identification of gene mutations involving epigenetic modifiers in paediatric AML is rare, accounting for approximately one tenth of those identified in adult AML[91]. However, comparison of the epigenetic landscape of paediatric and adult AML investigated by assessing promoter hypermethylation in a paediatric (n=70) and adult (n=383) cohort revealed that there was a non-random pattern of promoter hypermethylation in childhood AML that accompanied specific genetic aberrations. In paediatric samples there was a higher degree of methylation in CBF

leukaemia compared to 11q23 AML suggesting that promoter hypermethylation plays a part in the leukaemogenesis of CBF AML. This did not reach significance in the adult cohort, which may reflect the differences in 11q23 aberrations found in paediatric and adult AML.[101]. When taking into consideration the mutational data on epigenetic regulators in paediatric and adult AML however, the current strategies in precision therapies to target epigenetic regulators may not impact on AML across all ages. Further study into epigenetic regulators in the initiation and maintenance of paediatric AML are warranted.

There is evidence of chromosomal translocations occurring *in utero* including MLL rearrangements (discussed previously), t(8;21)[102] and case reports of inv(16) and t(15;17)[103]. Matched Guthrie cards from 12 patients with childhood AML with t(8;21) were screened. The *AML1-ETO* fusion transcript was detected in 50% of Guthrie cards proving that the translocation had originated *in utero* during fetal haematopoiesis. Interestingly, the oldest age of presentation of AML in a patient with evidence of genomic AML1-ETO at birth was over 12 years old[103]. Unlike MLL translocations which present early in life, it appears that prenatal t(8;21) can have a protracted latency to AML development. Furthermore, both AML1-ETO and PML-RARA transcripts can be detectable in patients post remission. In the case of AML1-ETO, transcripts are detectable in a fraction of stem cells, myeloid and B cells[104], implying that some but not all multipotent progenitor cells retain this translocation. This is in contrast to the age-related clonal haematopoiesis associated with DNMT3A mutations, in which all haematopoietic cells exhibit this mutation. AML harbouring AML1-ETO or PML-RARA has very good outcomes and low rates of relapse, suggesting that while a 'pre-leukaemic clone' is persistent, the leukaemogenicity, and therefore relapse rate is lower than the clonal haematopoiesis seen with age.

Although there are now several publications that document the frequency of somatic mutations occurring in epigenetic regulators expressed by healthy adults[78-80], no equivalent studies have been performed on healthy children, therefore it is hard to speculate if paediatric AML follows the same step-wise accumulation of mutations leading to the onset of disease. The age-related clonal evolution model has important clinical implications for the development of curative therapies for adult AML. The development of DNMT and TET2 inhibitors may provide a potential way to eradicate pre-leukaemic subclones, which are currently chemotherapy resistant[83] and need to be eradicated to avoid AML relapse. In contrast, the low incidence of these mutations in childhood AML suggests that

the pathogenic process of AML development and relapse is very different between paediatric and adult AML. Therefore, more studies are warranted to elucidate the mechanism of development of paediatric AML allowing treatment to be tailored to the pathogenic mechanisms.

Concluding remarks

There is growing evidence that cell ontogeny affects cellular biology with respect to epigenetic marks, gene expression and resultant cell characteristics. It therefore seems reasonable to hypothesise that the age of the cell will influence these cellular processes following an oncogenic insult. Evidence for the origins of many childhood AML associated translocations *in utero*, developing in a fetal stem cell implies different pathogenic mechanism are at play. This is in contrast to adult AML which has little evidence of a fetal origin but rather the generation of clonal haematopoiesis originating from an accumulation of somatic mutations in hematopoietic subclones. If pathogenesis is different, then treatment and particularly, development of novel therapies in childhood AML will be different to adult AML. Paediatric AML clearly has distinct biological properties compared to adult AML and extrapolating treatment from adult AML may not always be entirely appropriate. There is a need for more clinical trials dedicated to paediatric AML but in order to accrue enough participants, cooperation of multinational sites is required. Ideally, background rates of cancer-associated genetic abnormalities in healthy children will be carried out. This has obvious ethical considerations both in the collection of samples but also in investigating for such genetic anomalies in a population unable to give consent. Finally, further preclinical studies investigating the role of ontogeny in leukemogenesis will help determine if cell intrinsic factors contribute to the biological and clinical disparity between childhood and adult AML.

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Table 1. Summary of biological characteristics and genetic abnormalities by age

Figure 1. Biological changes in the HSC during normal aging and implications for leukaemic transformation

References

- [1] Howlader N NA, Krapcho M, Garshell J, Miller D, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2011. In: Institute. NC, ed. Bethesda, MD: National Cancer Institute. ; 2014.
- [2] Grimwade D, Walker H, Oliver F, et al. The Importance of Diagnostic Cytogenetics on Outcome in AML: Analysis of 1,612 Patients Entered Into the MRC AML 10 Trial. *Blood*. 1998;92:2322-2333.
- [3] Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. *Blood*. 2006;107:3481-3485.
- [4] Creutzig U, Zimmermann M, Bourquin JP, et al. Randomized trial comparing liposomal daunorubicin with idarubicin as induction for pediatric acute myeloid leukemia: results from Study AML-BFM 2004. *Blood*. 2013;122:37-43.
- [5] Juliusson G, Antunovic P, Derolf A, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood*. 2009;113:4179-4187.
- [6] Burnett AK, Hills RK, Milligan DW, et al. Attempts to optimize induction and consolidation treatment in acute myeloid leukemia: results of the MRC AML12 trial. *J Clin Oncol*. 2010;28:586-595.
- [7] Gibson BE, Webb DK, Howman AJ, et al. Results of a randomized trial in children with Acute Myeloid Leukaemia: medical research council AML12 trial. *Br J Haematol*. 2011;155:366-376.
- [8] Burnett A, Wetzler M, Lowenberg B. Therapeutic advances in acute myeloid leukemia. *J Clin Oncol*. 2011;29:487-494.
- [9] Creutzig U, Buchner T, Sauerland MC, et al. Significance of age in acute myeloid leukemia patients younger than 30 years: a common analysis of the pediatric trials AML-BFM 93/98 and the adult trials AMLCG 92/99 and AMLSG HD93/98A. *Cancer*. 2008;112:562-571.

- [10] Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114:937-951.
- [11] Creutzig U, van den Heuvel-Eibrink MM, Gibson B, et al. Diagnosis and management of acute myeloid leukemia in children and adolescents: recommendations from an international expert panel. *Blood*. 2012;120:3187-3205.
- [12] Harrison CJ, Hills RK, Moorman AV, et al. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment trials AML 10 and 12. *J Clin Oncol*. 2010;28:2674-2681.
- [13] Cornelissen JJ, van Putten WL, Verdonck LF, et al. Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom? *Blood*. 2007;109:3658-3666.
- [14] Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*. 2010;116:354-365.
- [15] Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 2010;115:453-474.
- [16] Niewerth D, Creutzig U, Bierings MB, Kaspers GJ. A review on allogeneic stem cell transplantation for newly diagnosed pediatric acute myeloid leukemia. *Blood*. 2010;116:2205-2214.
- [17] Byrd JC, Mrozek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*. 2002;100:4325-4336.

- [18] von Neuhoff C, Reinhardt D, Sander A, et al. Prognostic impact of specific chromosomal aberrations in a large group of pediatric patients with acute myeloid leukemia treated uniformly according to trial AML-BFM 98. *J Clin Oncol*. 2010;28:2682-2689.
- [19] Balgobind BV, Raimondi SC, Harbott J, et al. Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. *Blood*. 2009;114:2489-2496.
- [20] Hollink IH, van den Heuvel-Eibrink MM, Arentsen-Peters ST, et al. NUP98/NSD1 characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct HOX gene expression pattern. *Blood*. 2011;118:3645-3656.
- [21] Gruber TA, Larson Gedman A, Zhang J, et al. An Inv(16)(p13.3q24.3)-encoded CBFA2T3-GLIS2 fusion protein defines an aggressive subtype of pediatric acute megakaryoblastic leukemia. *Cancer Cell*. 2012;22:683-697.
- [22] Tosi S, Harbott J, Teigler-Schlegel A, et al. t(7;12)(q36;p13), a new recurrent translocation involving ETV6 in infant leukemia. *Genes Chromosomes Cancer*. 2000;29:325-332.
- [23] Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood*. 2001;98:1752-1759.
- [24] Meshinchi S, Woods WG, Stirewalt DL, et al. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. *Blood*. 2001;97:89-94.
- [25] Schneider F, Hoster E, Schneider S, et al. Age-dependent frequencies of NPM1 mutations and FLT3-ITD in patients with normal karyotype AML (NK-AML). *Ann Hematol*. 2012;91:9-18.
- [26] Thiede C, Koch S, Creutzig E, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). *Blood*. 2006;107:4011-4020.
- [27] Brown P, McIntyre E, Rau R, et al. The incidence and clinical significance of nucleophosmin mutations in childhood AML. *Blood*. 2007;110:979-985.

- [28] Hollink IH, Zwaan CM, Zimmermann M, et al. Favorable prognostic impact of NPM1 gene mutations in childhood acute myeloid leukemia, with emphasis on cytogenetically normal AML. *Leukemia*. 2009;23:262-270.
- [29] Green CL, Koo KK, Hills RK, Burnett AK, Linch DC, Gale RE. Prognostic significance of CEBPA mutations in a large cohort of younger adult patients with acute myeloid leukemia: impact of double CEBPA mutations and the interaction with FLT3 and NPM1 mutations. *J Clin Oncol*. 2010;28:2739-2747.
- [30] Ho PA, Alonzo TA, Gerbing RB, et al. Prevalence and prognostic implications of CEBPA mutations in pediatric acute myeloid leukemia (AML): a report from the Children's Oncology Group. *Blood*. 2009;113:6558-6566.
- [31] Taskesen E, Bullinger L, Corbacioglu A, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. *Blood*. 2011;117:2469-2475.
- [32] Ley TJ, Ding L, Walter MJ, et al. DNMT3A mutations in acute myeloid leukemia. *N Engl J Med*. 2010;363:2424-2433.
- [33] Thol F, Damm F, Ludeking A, et al. Incidence and prognostic influence of DNMT3A mutations in acute myeloid leukemia. *J Clin Oncol*. 2011;29:2889-2896.
- [34] Ho PA, Kutny MA, Alonzo TA, et al. Leukemic mutations in the methylation-associated genes DNMT3A and IDH2 are rare events in pediatric AML: a report from the Children's Oncology Group. *Pediatric blood & cancer*. 2011;57:204-209.
- [35] Thol F, Heuser M, Damm F, Klusmann JH, Reinhardt K, Reinhardt D. DNMT3A mutations are rare in childhood acute myeloid leukemia. *Haematologica*. 2011;96:1238-1240.
- [36] Ford AM, Ridge SA, Cabrera ME, et al. In utero rearrangements in the trithorax-related oncogene in infant leukaemias. *Nature*. 1993;363:358-360.

- [37] Gale KB, Ford AM, Repp R, et al. Backtracking leukemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. *Proc Natl Acad Sci U S A*. 1997;94:13950-13954.
- [38] Pine SR, Guo Q, Yin C, Jayabose S, Druschel CM, Sandoval C. Incidence and clinical implications of GATA1 mutations in newborns with Down syndrome. *Blood*. 2007;110:2128-2131.
- [39] Heald B, Hilden JM, Zbuk K, et al. Severe TMD/AMKL with GATA1 mutation in a stillborn fetus with Down syndrome. *Nature clinical practice Oncology*. 2007;4:433-438.
- [40] Zipursky A, Brown E, Christensen H, Sutherland R, Doyle J. Leukemia and/or myeloproliferative syndrome in neonates with Down syndrome. *Seminars in perinatology*. 1997;21:97-101.
- [41] Al-Kasim F, Doyle JJ, Massey GV, Weinstein HJ, Zipursky A, Pediatric Oncology G. Incidence and treatment of potentially lethal diseases in transient leukemia of Down syndrome: Pediatric Oncology Group Study. *Journal of pediatric hematology/oncology*. 2002;24:9-13.
- [42] Gurbuxani S, Vyas P, Crispino JD. Recent insights into the mechanisms of myeloid leukemogenesis in Down syndrome. *Blood*. 2004;103:399-406.
- [43] Massey GV, Zipursky A, Chang MN, et al. A prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): Children's Oncology Group (COG) study POG-9481. *Blood*. 2006;107:4606-4613.
- [44] Klusmann JH, Creutzig U, Zimmermann M, et al. Treatment and prognostic impact of transient leukemia in neonates with Down syndrome. *Blood*. 2008;111:2991-2998.
- [45] Gams AS, Alonzo TA, Gerbing RB, et al. Natural history of transient myeloproliferative disorder clinically diagnosed in Down syndrome neonates: a report from the Children's Oncology Group Study A2971. *Blood*. 2011;118:6752-6759; quiz 6996.
- [46] Ahmed M, Sternberg A, Hall G, et al. Natural history of GATA1 mutations in Down syndrome. *Blood*. 2004;103:2480-2489.
- [47] Chessells JM. Leukaemia in the young child. *The British journal of cancer Supplement*. 1992;18:S54-57.

- [48] Pui CH, Kane JR, Crist WM. Biology and treatment of infant leukemias. *Leukemia*. 1995;9:762-769.
- [49] Carroll A, Civin C, Schneider N, et al. The t(1;22) (p13;q13) is nonrandom and restricted to infants with acute megakaryoblastic leukemia: a Pediatric Oncology Group Study. *Blood*. 1991;78:748-752.
- [50] Creutzig U, Zimmermann M, Bourquin JP, et al. Favorable outcome in infants with AML after intensive first- and second-line treatment: an AML-BFM study group report. *Leukemia*. 2012;26:654-661.
- [51] Cozzio A, Passegue E, Ayton PM, Karsunky H, Cleary ML, Weissman IL. Similar MLL-associated leukemias arising from self-renewing stem cells and short-lived myeloid progenitors. *Genes Dev*. 2003;17:3029-3035.
- [52] Wang J, Iwasaki H, Krivtsov A, et al. Conditional MLL-CBP targets GMP and models therapy-related myeloproliferative disease. *EMBO J*. 2005;24:368-381.
- [53] Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. *Blood*. 2003;102:2395-2402.
- [54] Meyer C, Hofmann J, Burmeister T, et al. The MLL recombinome of acute leukemias in 2013. *Leukemia*. 2013;27:2165-2176.
- [55] Horton SJ, Jaques J, Woolthuis C, et al. MLL-AF9-mediated immortalization of human hematopoietic cells along different lineages changes during ontogeny. *Leukemia*. 2013;27:1116-1126.
- [56] Magee JA, Ikenoue T, Nakada D, Lee JY, Guan KL, Morrison SJ. Temporal changes in PTEN and mTORC2 regulation of hematopoietic stem cell self-renewal and leukemia suppression. *Cell Stem Cell*. 2012;11:415-428.

- [57] Babovic S, Eaves CJ. Hierarchical organization of fetal and adult hematopoietic stem cells. *Experimental cell research*. 2014;329:185-191.
- [58] Rebel VI, Miller CL, Eaves CJ, Lansdorp PM. The repopulation potential of fetal liver hematopoietic stem cells in mice exceeds that of their liver adult bone marrow counterparts. *Blood*. 1996;87:3500-3507.
- [59] Bowie MB, Kent DG, Dykstra B, et al. Identification of a new intrinsically timed developmental checkpoint that reprograms key hematopoietic stem cell properties. *Proc Natl Acad Sci U S A*. 2007;104:5878-5882.
- [60] Sudo K, Ema H, Morita Y, Nakauchi H. Age-associated characteristics of murine hematopoietic stem cells. *J Exp Med*. 2000;192:1273-1280.
- [61] Dykstra B, Olthof S, Schreuder J, Ritsema M, de Haan G. Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. *J Exp Med*. 2011;208:2691-2703.
- [62] Benz C, Copley MR, Kent DG, et al. Hematopoietic stem cell subtypes expand differentially during development and display distinct lymphopoietic programs. *Cell Stem Cell*. 2012;10:273-283.
- [63] McKinney-Freeman S, Cahan P, Li H, et al. The transcriptional landscape of hematopoietic stem cell ontogeny. *Cell Stem Cell*. 2012;11:701-714.
- [64] Sun D, Luo M, Jeong M, et al. Epigenomic Profiling of Young and Aged HSCs Reveals Concerted Changes during Aging that Reinforce Self-Renewal. *Cell Stem Cell*. 2014;14:673-688.
- [65] Flach J, Bakker ST, Mohrin M, et al. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. *Nature*. 2014;512:198-202.
- [66] Santos MA, Faryabi RB, Ergen AV, et al. DNA-damage-induced differentiation of leukaemic cells as an anti-cancer barrier. *Nature*. 2014;514:107-111.
- [67] Holyoake TL, Nicolini FE, Eaves CJ. Functional differences between transplantable human hematopoietic stem cells from fetal liver, cord blood, and adult marrow. *Exp Hematol*. 1999;27:1418-1427.

- [68] Pang WW, Price EA, Sahoo D, et al. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. *Proc Natl Acad Sci U S A*. 2011;108:20012-20017.
- [69] Rufer N, Brummendorf TH, Dragowska V, Shultz M, Wadsworth LD, Lansdorf PM. Turnover of stem cells, naive and memory T lymphocytes, estimated from telomere fluorescence measurements. *Cytotherapy*. 1999;1:342.
- [70] Sidorov I, Kimura M, Yashin A, Aviv A. Leukocyte telomere dynamics and human hematopoietic stem cell kinetics during somatic growth. *Exp Hematol*. 2009;37:514-524.
- [71] Rube CE, Fricke A, Widmann TA, et al. Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging. *PLoS One*. 2011;6:e17487.
- [72] Welch JS, Ley TJ, Link DC, et al. The origin and evolution of mutations in acute myeloid leukemia. *Cell*. 2012;150:264-278.
- [73] Valk PJ, Verhaak RG, Beijen MA, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. *N Engl J Med*. 2004;350:1617-1628.
- [74] Haferlach T, Kohlmann A, Wiczorek L, et al. Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. *J Clin Oncol*. 2010;28:2529-2537.
- [75] de Jonge HJ, Valk PJ, Veeger NJ, et al. High VEGFC expression is associated with unique gene expression profiles and predicts adverse prognosis in pediatric and adult acute myeloid leukemia. *Blood*. 2010;116:1747-1754.
- [76] Ross ME, Mahfouz R, Onciu M, et al. Gene expression profiling of pediatric acute myelogenous leukemia. *Blood*. 2004;104:3679-3687.
- [77] Balgobind BV, Van den Heuvel-Eibrink MM, De Menezes RX, et al. Evaluation of gene expression signatures predictive of cytogenetic and molecular subtypes of pediatric acute myeloid leukemia. *Haematologica*. 2011;96:221-230.

- [78] Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371:2477-2487.
- [79] Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371:2488-2498.
- [80] Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med*. 2014;20:1472-1478.
- [81] Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature*. 2014;506:328-333.
- [82] Jan M, Snyder TM, Corces-Zimmerman MR, et al. Clonal evolution of preleukemic hematopoietic stem cells precedes human acute myeloid leukemia. *Science translational medicine*. 2012;4:149ra118.
- [83] Corces-Zimmerman MR, Hong WJ, Weissman IL, Medeiros BC, Majeti R. Preleukemic mutations in human acute myeloid leukemia affect epigenetic regulators and persist in remission. *Proc Natl Acad Sci U S A*. 2014;111:2548-2553.
- [84] Majeti R. Clonal evolution of pre-leukemic hematopoietic stem cells precedes human acute myeloid leukemia. *Best Pract Res Clin Haematol*. 2014;27:229-234.
- [85] Chan SM, Majeti R. Role of DNMT3A, TET2, and IDH1/2 mutations in pre-leukemic stem cells in acute myeloid leukemia. *Int J Hematol*. 2013;98:648-657.
- [86] Tie R, Zhang T, Fu H, et al. Association between DNMT3A mutations and prognosis of adults with de novo acute myeloid leukemia: a systematic review and meta-analysis. *PLoS One*. 2014;9:e93353.
- [87] Yan XJ, Xu J, Gu ZH, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. *Nature genetics*. 2011;43:309-315.
- [88] Yamashita Y, Yuan J, Suetake I, et al. Array-based genomic resequencing of human leukemia. *Oncogene*. 2010;29:3723-3731.

- [89] Mardis ER, Ding L, Dooling DJ, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med.* 2009;361:1058-1066.
- [90] Thol F, Friesen I, Damm F, et al. Prognostic significance of ASXL1 mutations in patients with myelodysplastic syndromes. *J Clin Oncol.* 2011;29:2499-2506.
- [91] Liang DC, Liu HC, Yang CP, et al. Cooperating gene mutations in childhood acute myeloid leukemia with special reference on mutations of ASXL1, TET2, IDH1, IDH2, and DNMT3A. *Blood.* 2013;121:2988-2995.
- [92] Patel JP, Gonen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med.* 2012;366:1079-1089.
- [93] Abdel-Wahab O, Levine RL. Mutations in epigenetic modifiers in the pathogenesis and therapy of acute myeloid leukemia. *Blood.* 2013;121:3563-3572.
- [94] Gaidzik VI, Paschka P, Spath D, et al. TET2 mutations in acute myeloid leukemia (AML): results from a comprehensive genetic and clinical analysis of the AML study group. *J Clin Oncol.* 2012;30:1350-1357.
- [95] Metzeler KH, Maharry K, Radmacher MD, et al. TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol.* 2011;29:1373-1381.
- [96] Weissmann S, Alpermann T, Grossmann V, et al. Landscape of TET2 mutations in acute myeloid leukemia. *Leukemia.* 2012;26:934-942.
- [97] Metzeler KH, Becker H, Maharry K, et al. ASXL1 mutations identify a high-risk subgroup of older patients with primary cytogenetically normal AML within the ELN Favorable genetic category. *Blood.* 2011;118:6920-6929.
- [98] Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nature genetics.* 2012;44:1179-1181.
- [99] Huether R, Dong L, Chen X, et al. The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. *Nature communications.* 2014;5:3630.

- [100] Ribeiro AF, Pratcorona M, Erpelinck-Verschueren C, et al. Mutant DNMT3A: a marker of poor prognosis in acute myeloid leukemia. *Blood*. 2012;119:5824-5831.
- [101] Juhl-Christensen C, Ommen HB, Aggerholm A, et al. Genetic and epigenetic similarities and differences between childhood and adult AML. *Pediatric blood & cancer*. 2012;58:525-531.
- [102] Wiemels JL, Xiao Z, Buffler PA, et al. In utero origin of t(8;21) AML1-ETO translocations in childhood acute myeloid leukemia. *Blood*. 2002;99:3801-3805.
- [103] Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. *Nature reviews Cancer*. 2003;3:639-649.
- [104] Miyamoto T, Weissman IL, Akashi K. AML1/ETO-expressing nonleukemic stem cells in acute myelogenous leukemia with 8;21 chromosomal translocation. *Proc Natl Acad Sci U S A*. 2000;97:7521-7526.