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EDITORIAL

Less Utilized Prognostic Markers in Acute Myeloid Leukemia

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ABSTRACT

Acute myeloid leukemia (AML) results from the over-proliferation of progenitor cells of the myeloid lineage in the bone marrow. It is a heterogeneous disease that is aggressive and difficult to treat. AML diagnosis is based on clinical as well as laboratory investigations. Risk stratification is important to assess risk of relapse. Current guidelines for risk stratification are dependent on identification of genetic aberrations particularly chromosomal translocations and gene mutations. Though these are observed in the majority of patients, the best treatment regimen remains elusive. AML blasts are assumed to have transformed from a normal counterpart and maintains many normal regulatory functions. Early features such as stem cell properties has long been proven to be linked to early relapse in AML. Leukaemia stem cells (LSC) are identified by high CD34 and negative CD38 expression. Aberrant expression of a third marker such as Thy-1/CD90 negativity, expression of CLL-1 and IL-3R (CD123), intermediate levels of aldehyde dehydrogenase and co-expression of common chromosomal translocation may be used to distinguish from normal hematopoietic stem cells. In vitro characteristics of AML blasts such as proliferation, survival or response to treatment are also able to predict patient response to therapy, replicative of its interaction with the microenvironment. These potential prognostic markers are well studied but are currently not considered in patient management.

Key words: Acute myeloid leukaemia; Leukaemia stem cell; In vitro characteristic; Prognostication

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INTRODUCTION

Acute myeloid leukemia (AML) is a hematological malignancy diagnosed by the presence of 20% or more blasts in the peripheral blood or bone marrow. Median age of presentation is 72 years (16-97 years old). AML is still relatively difficult to treat. Early death (death within 30 days from diagnosis), excluding acute promyelocytic leukemia occurs in 19% of patients. Complete remission is achievable in 65% de novo AML and 41% secondary AML. Median overall survival for these patients was 1119 days for age 16-55 years (*N*=554), 350 days for age 56-65 years (*N* = 437) and 80 days for patients aged 76-89 year old (*N* = 968)[1].

The French-American-British (FAB) classification of acute myeloid leukemias, the first classification established more than 40 years ago, identifies eight subtypes (M0-M7) based on morphology and cytochemistry staining.

The WHO classification of AML is now used worldwide and provide guidelines for diagnosis as well as risk stratification. This classification utilizes multidisciplinary approaches and all available information including morphologic, cytochemical, immunophenotypic, clinical information as well as genetics in the diagnostic algorithm[2].

Risk stratification begins immediately as it guides patient's treatment regimen. Prognostic markers predict treatment related mortality (TRM), resistance to chemotherapy or survival. TRM relates to patient characteristics and general health while drug resistance or overall survival is characteristic of the AML clone. Patient related factors such as age, performance status, co-morbidities and AML-related factors such as white blood count, transformation from MDS , therapy-related AML, genetic abnormalities all influence patient's treatment outcome (reviewed in Dohner et al[3], 2010).

Recurring cytogenetic abnormalities is the single most valuable prognostic factor in AML and is able to categorize patients into:

(1) Favorable: those with t (8;21); RUNX1-RUNX1T1, inv (16); CBFB-MYH11 or t (15;17); PML-RARA;

(2) Intermediate: those with no abnormality/ cytogenetic normal (CN), +8, 11q23, +21, del(7q), del (9q), +22, or other numerical and structural changes;

(3) Unfavorable: complex, -7, abn(3q), del(5q) or -5.

Translocations t(8;21), t(15;17), inv(16)/t(16;16) and 11q23 constitute 34% to 47% of pediatric and 21% to 28% of adult cytogenetic abnormalities. Trisomies, deletions and complex karyotypes contribute to another large percentage of adult AMLs[4].

While these abnormalities have prognostic significance, many do not correlate with FAB classification. Translocation t(8;21)/AML1 (RUNX1)-ETO is one of the most common karyotypic abnormalities in AML and confers a favorable prognosis. It is also predominantly, but not exclusively, associated with AML M2. AML1-ETO was found in 3% (1/32) of AML M1 and 25% (8/32) of M2[5]. Others found this translocation in 12.5%[6] and 22%[7] of M2.

Approximately, 40-42% present with normal cytogenetics, CN-AML[8,9] The latest WHO classification (2008) recommends mutational studies for NPM1, CEBPA, and FLT3 in cytogenetically normal AML. Other important diagnostic and prognostic markers in AML are RUNX1, KIT, WT1, and MLL[2].

"Mutated NPM1 is found in 50% to 60% of cases of cytogenetically normal AML, and when only NPM1 mutation status is considered, approximately 50% survival at 4 years is observed. FLT3-ITD mutation is found in approximately 30% to 40% of cases of cytogenetically normal AML, and when only the FLT3-ITD status is considered, survival rate of only 20% to 25% at 4 years was achieved. Mutations of CEBPA are found in approximately 15% of cytogenetically normal AML cases and these patients have a nearly 60% 4-year survival rate. When NPM1 and FLT3-ITD status are considered together, patients who have mutated NPM1 and are FLT3-ITD–negative have a 4-year survival similar to that of the CEBPA-mutated cases at approximately 60%, whereas the remaining cases, being either NPM1 wild-type or FLT3-ITD–positive or both, have a dismal 30% survival rate at 4 years" (extracted from[2]). KIT mutation is common in core-binding factor AML, that is, those with rearranged RUNX1 and CBFB genes. However, a higher relapse rate and lower overall survival is noted when mutated KIT is present[2].

By integrating mutational studies such as internal tandem duplication in FLT3 (FLT3-ITD), partial tandem duplication in MLL (MLL-PTD) and mutations in ASX1 and PHF6, CEBPA, IDH1, IDH2 and NPM1, the intermediate category (forming 63%) could be realigned, increasing the favorable category (initially 19%) to 26% and the unfavorable category (initially 18%) to 39%. The percentage in the intermediate group drops to 35%. Three-year overall survival for favourable, intermediate and unfavourable were achieved in 85%, 42% and 13%, respectively[10].

The European LeukemiaNet is represented by an international expert panel to provide updated recommendations for the diagnosis and management of AML in adults (excluding acute promyelocytic leukemia). To better compare data among studies, a standardized reporting system for genetic abnormalities was proposed which includes data from cytogenetic analysis and mutational analyses of NPM1, CEBPA and FLT3 genes. Based on these genetic changes, the European LeukemiaNet established the Favorable, Intermediate I, Intermediate II and Adverse risk groups[3].

Current conventional treatment of AML is still largely chemotherapy-based. Treatment starts with induction therapy which consists of intensive therapy with anthracycline and cytarabine given to all patients except older patients (>60 yr) with adverse cytogenetic risk. The purpose of induction therapy is to achieve complete remission (CR). Postremission therapy for patients with favourable genetic risk is consolidation therapy of intermediate dose cytarabine. Cure rates among these patients are 60-70%.

Patients with intermediate or adverse risk are considered for allogeneic hematopoietic cell transplantation (HCT) if a donor is available. A HLA-matched graft is generally preferred. The HCT procedure provides the strongest antineoplastic therapy from pretransplantation conditioning and the immunologic antileukemic graft-versus-leukemia effect. Even though patients with intermediate-risk genetic factors may fare better, without HCT the outcome remains poor with cure rates of only 10-15%. The outcomes in patients who are older than 60 years of age remain highly unsatisfactory[11].

Relapse rates in AML patients <60 years old in complete response according to the European LeukemiaNet risk groups of good, intermediate, poor and very poor were 35-40%, 50-55%, 70-80% and >90%, respectively. HSCT is expected to reduce relapse rate in these patients by half[12]. Autologous hematopoietic stem cell transplantation is considered an alternative option for postremission therapy in patients with favourable- and intermediate-risk cytogenetics, whereas it cannot be recommended in patients with high-risk cytogenetics[3].

Postremission monitoring of minimal residual disease (using quantitative polymerase chain reaction) initiates quick response to provide salvage therapy including allogeneic hematopoietic cell transplantation by molecular detection of persistent or relapse AML to improve outcome.

Other than cytotoxic chemotherapy and stem cell transplantation, risk stratification based on cytogenetics and molecular markers which provides the means to assess risk of relapse, has made it possible for targeted therapy. Recurring genetic changes affecting various genes including NPM1, CEBPA, RUNX1, MLL, FLT3, KIT and RAS are recommended for inclusion in diagnosis. However, these genetic aberrations do not encompass all patients nor represent all changes found in AML. In fact, many other genetic changes have been identified including epigenetic changes[13]. Furthermore, mutational changes continue to occur between a patient's diagnosis and relapse. The uniqueness of these aberrant patterns to each patient has made it apparent that successful therapy will depend on personalized treatment most likely in combination with conventional chemotherapy.

The two-hit model of leukaemogenesis identifies two classes of mutations where class I mutations in tyrosine kinases results in enhanced proliferation or survival (e.g. FLT3-ITD, FLT3-TKD, KIT, RAS, PTPN11, JAK2) while class II resulting from loss of function of transcription factors and needed for differentiation, effects a block in differentiation (e.g. PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11, MLL fusions, CEBPA and may be NPM1)[14]. While not all have drugs developed, a large number of targeted drugs with anti-cancer potential are available. Ex vivo testing on AML samples indicated tyrosine kinase inhibitors, MAP-ERK kinase inhibitors, rapalogs, as well as AKT inhibitors and mTOR inhibitors are potentially suitable[15].

However, targeted therapy is not without challenges. It is increasingly evident that many molecular targets may not, in the end, be clinically relevant. Targets that are truly critical to the survival of the cell turned out to be not the only check point as cancer cells are able to escape by choosing an alternative pathway. Testing

of multiple molecular targets may be an option but will need to circumvent challenges in designing clinical trials with more than a single drug company within the same study. Presently, it is not clear how the safety and efficacy of such highly personalized therapies can be appropriately assessed when taken to its extreme, personalized therapy will ultimately result in sample size of only 1[16].

Historically, investigations on mechanisms of relapse or drug resistance extended beyond genetic aberrations and included biological characteristics that also provided significant clinical impact. The following discusses several of these markers.

EARLY PROGENITORS AND LEUKEMIA STEM CELLS

The 2008 WHO classification includes a category as AML, not otherwise specified (NOS), which is based on morphology/ cytochemistry and estimated to account for 25%-30% of all cases[2]. Cases with history of myelodysplastic syndrome, simultaneous evidence of myelodysplasia or complex and unbalanced cytogenetic changes are excluded from this grouping[10].

Subcategories here are similar to the earlier French-British-American classification of AML based on cell maturity i.e. AML with minimal differentiation (M0); AML without maturation (M1); AML with maturation (M2); acute myelomonocytic leukemia (M4) and acute monoblastic/monocytic leukemia (M5). In AML cases, distribution of these subtypes were M0 (mean 6%, range 5%-8%), M1 (25%, 16%-27%), M2 (28%, 27%-34%), M4 (21%, 13%-27%), M5 (15%, 12%-26%) including M6 and M7, excluding M3 [17]. Thus, the earliest progenitor candidate is predicted to be M0, forming the smallest subcategory.

M0 is diagnosed by less than 3% myeloperoxidase/Sudan Black B by cytochemistry and immunophenotyped positive for CD13 and or CD33 but negative for B and T lineage markers[18]. NPM1 mutation is not observed in FAB M0 type[19]. FLT3/ITD mutations are also not observed in M0 type (FAB classification) but more frequent in the other groups in particular M3[20]. CEBPA mutations, an independent prognostic factor for improved outcome in AML, are significantly more common in older patients and patients with FAB M1 and M2 and normal karyotype but not observed in FAB M0[21,22]. A total of 45%-71% of M0 have normal cytogenetics in contrast to 28% in M3 and 64% in M4[7].

The M0 subgroup was found to have a significantly lower 5-year survival rate (20.8%) when this FAB type was compared with M1 (32.2%), M2 (38.9%) M3 (61.5%), M4 (26.4%), M4Eo (56%) and M5 (22%)[4]. With the newer classification, M0 cases achieved 65% complete remission, 8% 5-year survival rates and median overall survival was 10.6 months[10]. In a larger study, among the FAB subgroups, complete remission was achieved in 63% (M0), 74% M1, 78%M2, 76% M4, and 80% M5 cases[17]. After multivariate adjustment, FAB M0 was independently associated with worse overall survival (OS) relative to M1, M2, M4, and M5[17]. Thus, the early progenitor subgroup appears to have a poorer outcome compared to others. However, early progenitor features are not limited to this group.

Precursor markers used in the diagnosis of AML include the stem cell marker CD34, CD38, CD117, CD133, HLA-DR, as recommended by European LeukemiaNet (ELN) 2010 for immunophenotyping and diagnosis of early cells[3]. Expression of CD34 and HLA-DR ranged from 83% to 100% M0 cases. CD117, a protein preferentially expressed on immature myeloid leukemia cells[23], ranged from 50% to 80% in M0 cases[23-26].

Studies comparing expression of these markers showed CD34 was expressed in the majority of FAB subtypes, 96% (M0), 79% (M1), 71% (M2), 71% (M4) with lesser cases in M3 (14%) and M5 (47%)[27]. Another study showed CD34, HLA-DR and CD117 were expressed in 100%, 100% and 75% of M0; 71%, 66% and 69% of M1; 82%, 86% and 89% of M2; 57%, 94% and 80% of M4; 86%, 100% and 100% of M4Eo and 20%, 100% and 16% of M5, respectively[28]. These indicate many cases in the subroups express early markers. CD133 expression correlated with CD34 but not FAB subtype[29]. CD133 was also significantly correlated with CD13, HLA-DR, CD7, CD3 and TdT[30].

Studies on prognostic value showed expression of CD34 correlated with lower rates of complete remission in AML. HLA-DR and CD117 expressions also correlated with reduced complete remission rates (reviewed in Mason *et al*[31], 2006). Initial study on CD133 expression in AML patients (n=102) concluded CD133 was not useful as a prognostic marker in AML[29]. Another study however, observed in AML (n=30) with CD133 expressions were associated with a significantly lower overall survival[30].

Expression patterns among risk groups observed CD34, HLA-DR and CD117 expressed in 95%, 100% and 68% of Good; 57%, 82%and 75% of Intermediate and 81%, 87% and 78% of Poor risk group[28].

Thus, undifferentiated AML blasts cells have a poorer outcome but the early feature of these cells are not limited to the M0 subgroup. Furthermore, expression of early markers is associated with poor outcome and are also dominant in other subgroups. Thus, another more suitable entity is desired.

Leukemia stem cell (LSC) was the first cancer stem cell to be described[32]. Xenotransplantation is the gold standard model for characterizing leukemic stem cell where leukemia initiating cells (LIC) have the potential for self-renewal. LIC induces AML in non-obese diabetic mice with severe combined immunodeficiency disease (NOD/SCID) and is termed SCID-IC. SCID-IC were exclusively CD34++CD38-[33]. In clinical settings, inclusion of CD34, CD38, CD117, CD133, HLA-DR are not mandatory in the AML diagnosis panel[3]. The CD34+CD38- pattern of expression was not reported in many of the studies on M0[10,23-27,34,35].

CD34+CD38- cells were observed in all subtypes of AML analysed[33]. However, cases negative for CD38 are low; 12.5% (M0), 15.5% (M1), 13.8% (M2), 4.2% (M3), 37.5% (M4) and 25% (M5) [36] where positive cases have >20% positive blasts detected. Among 56 AML analyzed, only one case fitted the CD34+CD38- profile and was of the FAB M4 subtype[37].

Nevertheless, AML cells from various FAB subtypes (M1, M2, M4 and M5) engrafted at high levels in the SCID- leukemia mice reproduced FAB morphological and features characteristic of the donor's disease[32]. Immature leukaemic blast colony-forming units (AML-CFU), detected in in vitro cultures, were present in all donor samples regardless of FAB classification, after transplantation in SCID mice. A search for a leukemia stem cell more immature than the AML-CFU determined that only the CD34+CD38- fraction gave rise to leukemia in transplanted mice[32]. Various other studies supported the presence of LSC in all FAB subtypes[38,39] with as much as 60%, 44%, 58% and 67% in M1, M2, M4 and M5 cases, respectively[40].

These dormant immature cells with high CD34+(CD38-) expression are thought to cause early relapse which contributes to poor survival[41] due to failure of current therapies to adequately target LSC[42]. Higher frequencies of CD34+CD38- LSC at diagnosis correlated with poor survival in both adult[41] and paediatric[43] AML.

The SCID-LIC however, shares the CD34+CD38- expression pattern similar to normal stem cells. Thus, various studies have identified markers that differentiated these two groups including lack of expression of Thy-1/CD90[38], expression of CLL-1[39] and IL-3R (CD123)[44], intermediate levels of aldehyde dehydrogenase[42] and co-expression of common chromosomal translocation[40] in the AML cases. These new phenotypes correlated with poor survival[39,40,42,44].

Global gene expression studies on LSC were designed to identify unique signatures of prognostic value. However, the LSC were largely derived from murine models induced with Meis1 and Hoxa9[20,45] compared between CD34+CD38- with CD34+CD38+ blasts within the AML cases[46] or compared with normal CD34+CD38- hematopoietic stem cells, HSC[47,48]. Comprehensive transcriptomic analysis to uncover genetic programs that maintain LSC in the self-renewing compartments in AML revealed gene expression patterns more akin to embryonic rather than adult stem cells. This was observed in the murine models of human leukemia induced by MLL-AF9 oncogene[49] as well as other cancers associated with poor prognosis (reviewed in Cleary, 2009[50]). Thus, LSCs which are considered to originate from hematopoietic stem or progenitor cells, not only adopt the regulatory machinery operating in normal HSCs but also establish their own mechanisms against apoptosis and senescence. Literature on the genetics of LSC remains few.

IN VITRO CHARACTERISTICS OF AML BLASTS

LSC cells engrafting NOD/SCID mice were also found to be the very cells that populate long term culture systems[38]. Self-renewal potential of LSC is demonstrable by colony forming blasts units (CFU-blast). CFUs were achieved by growing samples on semi-solid methylcellulose culture system supplemented with growth factors such as EPO, IL-3, IL-6, G-CSF, GM-CSF and Steel Factor[38] or with PHA-LCM. Whole native AML population or selected cells sorted or purified based on cell surface antigen expression may be used in cultures.

Cell suspension cultures (with or without GF autocrine) and pre-established irradiated human marrow feeders (with or without added exogenous growth factors) were other culture conditions to maintain LSCs. Suspension culture assays may also be carried out in serum free medium with similar recombinant growth factors[38]. Duration of cultures may be long-term, from 2-8 weeks or short-term, up to 7 days. These methods were used to observe for spontaneous proliferation or spontaneous apoptosis. Some patients' samples exhibited spontaneous proliferation while others can only proliferate in the presence of exogenous growth factors (reviewed in Bruserud *et al*[51], 2001).

By maintaining cells for 4 weeks or longer in suspension culture in the presence of growth factors or culture on stroma, leukemia progenitor with higher proliferative potential could be separated. Self-renewal potential were then determined by CFU counts which showed heterogeneity and progenitor hierarchy among AML samples[52].

The capacity for self-renewal in AML clones was shown early on as an important contributor to outcome in AML including successful remission induction and duration of survival. The number of CFU-blast induced from AML cells depleted of T cells and stimulated with PHA-L[53,54] or by autonomous growth[54] was inversely correlated with remission induction[53,55] and duration of survival[53,54,55].

AML blasts in suspension cultures also utilized other detection methods including incorporation of tritiated thymidine incorporation[56] and methyl tetrazolium (MTT) assay[57,58] to determine rate of proliferation and survival, respectively. High levels correlated with poor response to chemotherapy[56,57] and short duration disease free survival[56,58]. Contrasting results were obtained in another study where spontaneous proliferative determined by tritiated thymidine incorporation was significantly higher in favourable karyotypes compared to cases with unfavorable cytogenetics[59]. This group used IMDM supplemented with fetal calf serum (FCS) as culture media. Lowenberg *et al*[56] (1993) and our group[57] used serum free media while Norgaard *et al*[57] (1996) used RPMI with FCS as culture media. The results here did not correlate with FAB subgroups.

Thus, experimental models that utilize short-term culture of total native AML populations are still important and relevant in the study of AML and they are also the experimental models with the best documented clinical relevance[51].

Recently, a mathematical model was developed to assess the properties of LSC in leukemia samples. The proliferative, apoptotic and self-renewal principles of normal and leukaemic progenitors at the levels of hematopoietic stem cells (HSC), hematopoietic progenitor cell (HPC) and postmitotic mature cell and their regulation and responses to cytokines were the basis of the model. LPC was assumed to proliferate faster than LSC while LSC had higher self-renewal rates. The system was applied to the clinical data of 41 patients with relapsed AML[60]. As expected, considerable inter-individual heterogeneity of LSC properties was observed among patients. More importantly, high self-renewal rate was required for leukemia relapse whereas fast proliferation rate was not always required. Nevertheless, LPCs play a major role as they speed up production of leukemic blasts[60]. Other mathematical models has been established to characterize stem cells based on signaling interaction with the environment[61] and determine how stem cells coordinate homeostasis through cross-talk between genetic and epigenetic regulation[62]. Mathematical and computational techniques were also used to gain insights into the biology of cancer stem cells in initiation, progression and response to treatment in chronic myeloid leukaemia (reviewed in Michor, 2008[63]), to determine how mutations deregulate stem cell homeostasis and induces cancer[64], how kinetics of cancer stem cell sustain growth[65] and to strategise how to eliminate cancer stem cells[66]. The lesser requirement for resources and lower complexity compared to clinical trials and laboratory procedures may make mathematical modeling a niche to guide in drug selection and prediction of treatment outcome in personalized medicine.

The balance between renewal and differentiation can be altered by exposing blast populations to various growth factors in culture[67]. In vitro assays provide an excellent method to screen for potential agents with differentiation induction potential. The best example was the use of all-trans retinoic acid (ATRA) on acute pro-myelocytic leukemia which also sensitized these cells to intensive chemotherapy. Other agents such as cytokines also induces leukaemic cell differentiation demonstrated in cells cultured in vitro, making it a promising therapeutic approach (reviewed in Bruserud *et al*[68], 2000). Various chemotherapeutic drugs target cells in division. Proliferative activity of AML blasts were significantly higher in favourable karyotypes compared to unfavourable karyotypes. Exposure to GM-CSF significantly increased proliferation in normal and unfavarouble groups compared to favourable karyotype[59].

CD34+ expression of cells from AML bone marrow were significantly reduced following incubation with a cytokine mix without affecting viability in all samples analysed. In contrast, this cytokine mix increased CD34-positive cells from all healthy donors

tested. The cytokine mix consisted of interleukin (IL)-1beta, IL-3, IL-6, stem cell factor (SCF), erythropoietin (EP) with granulocyte macrophage/colony-stimulating factor (GM-CSF)[69]. Gemtuzumab ozogamicin (Mylotarg) resulted in a 34% reduction in CD34+CD38-CD123+ LSPC number, whereas normal CD34+CD38- hemapoietic stem cells were insensitive to the agent[70]. Another agent, protein kinase C agonist prostratin induced differentiation of human myeloid leukemia cells and enhanced cellular differentiation by chemotherapeutic agents[71].

Other examples of differentiation inducing agents include: (1) vitamin analogs: retinoids and vitamin D derivatives (2) cytokines: granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), interferons, tumor necrosis factor (TNF) (3) polar-aplanar compounds: hexamethylene bisacetate (HMBA) (4) histone deacetylase inhibitors: trichostatin (TSA), phenylbutyrate,61 apicidin,62 depsipeptide (FR901228)63 (5) inhibitors of DNA methylation: 5-aza-2′deoxycytidine64 (6) cyclic AMP analogs: 8-Cl-cAMP, dibutyl cAMP (7) chemotherapeutic agents: aclarubicin, cytosine arabinoside (Ara-C), hydroxyurea and even (8) medicinal plant-derived products and plant growth regulators (reviewed in Wang *et al*[72], 2003).

A drug sensitivity test performed in the laboratory prior to clinical treatment, similar to the approach of antibiotic susceptibility testing in microbiological disease, has long been an aspiration in AML. Pemovska *et al*[15] (2013) tested drugs used in conventional chemotherapies including daunorubicin, idarubicin, cytarabine and also molecularly targeted drugs already tested and approved for non-AML indications (eg: Dasatinib, Trametinib and Temsirolimus) and 64 new investigational agents (eg: Foretinib, Dactolisib and MK-2206).

Combining genomic studies and ex vivo drug sensitivity and resistance testing (DSRT) made it possible to not only identify the most appropriate drug but also its anti-cancer selectivity from testing on normal cells. Deep molecular profiling uncovered mechanisms of drug response and resistance by monitoring therapy responses at the level of individual AML subclones. Despite heterogeneity in genotype and phenotype, similar sensitivity drug patterns for certain drug classes were observed in these AML samples. Thus, in vitro characterization may also assist in challenges faced in personalized treatment[15].

EARLY POST-INDUCTION MARKERS

Detecting early signs of chemo-resistance is valuable for successful remission induction. Many studies have reported delayed clearance of blasts is predictive of a worse prognosis (discussed in Rowe *et al*[73], 2010). Residual leukemia present on day 14 bone marrow can be an early indicator of a highly resistant clone. However, it may also represent a slower response to therapy. It is standard practice in some groups to repeat an identical course of induction therapy at that point. Long-term outcome was similar to the group achieving complete remission with one cycle even when given identical post-remission therapy[73].

Identifying residual blasts is subjective to the examiner as morphological appearances can be difficult to interpret. Detection of minimal residual disease (MRD) is currently recommended in the management of AML, however the best method to use to measure MRD is still a matter of debate[74]. The application of MRD will not be discussed here as there are many publications on this subject.

Specific and sensitive markers detected post-induction are being investigated. Wilms' tumor gene 1 (WT1) is a gene commonly over-expressed in approximately 45% of AML cases. Quantitative analysis of bone marrow levels differentiated three groups where lower copies were associated with better treatment outcome[75]. PRAME is another potentially important marker for MRD monitoring[76]. Its value in early post-induction is still unknown. Bone marrow evaluation of cytokine transcripts after 5 days induction therapy indicated interleukin-1β in particular may be able to identify AML resistant to chemotherapy[77].

Assessing bone marrow however, is invasive and rather painful. Methods that could reduce this need would be advantageous to patient comfort. WT1 mRNA levels in peripheral blood of patients after achieving complete remission predicted relapse after CR[78]. Studies evaluating specific markers expressed in the peripheral blood early during induction therapy are not available. Our study indicated the potential of phosphorylated-Bad, a signaling mediator of the MAPK and Akt pathways in identifying AML resistant to chemotherapy early during induction therapy, day 3- day 7[79].

CONCLUSION

Thus, various factors remain that are not considered in diagnosis or prognostication of AML. Of concern is current classification provide researchers with very little basis to move forward in understanding the biology of AML. Convincing results exists for the role of LSC in inducing early relapse. Laboratory diagnosis should include detection of the CD34+D38- population and a supporting aberrant marker. Clinical trials could be conducted to tailor inclusion of a differentiation agent.

Relapse is not only dependent on estimation of the total number of LSC at diagnosis but may be influenced by the dynamics of LSC and interaction with its microenvironment. Chemotherapy may alter the microenvironment accelerating the return of a small number of surviving LSC or may cause it to remain dormant after cessation of therapy implied by division kinetics as well as self-renewal rates of these cells. In vitro cultures of AML blasts in the right media and growth factors may be used to select for the best therapy to target the unique biology of the cells. There is suggestion that both drugs and growth factors should be assessed for their effects on self-renewal as part of preclinical testing[67].

Mathematical approaches will benefit by advancing the effort to learn more of the hematopoietic system and its diseases in advance of available experimental methods and may be useful as a tool for personalized medicine.

CONFLICT OF INTEREST

The author declare no conflicts of interest.

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