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Review Article

A Review Article on Inherited Leukemias.

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Introduction

Leukaemia is a type of blood cancer and arises as a result of uncontrolled expansion of immature bone marrow cells that have failed to mature and differentiate. The human body constantly produces new blood cells in a finely balanced process known as haematopoiesis. This process begins with stem cells that divide to produce blast cells that develop into immature blood cells. Immature blood cells differentiate to become either lymphoid or myeloid cells. Lymphoid cells include B and T lymphocytes and are white blood cells that provide an immune response that combat infections (Figure 1). Myeloid cells differentiate into other blood components such as red blood cells, platelets, monocytes and granulocytes. (Engblom, Pfirschke and Pittet, 2020). Hence leukaemia can develops in any cell lineage in which there is a dysfunctional haematopoiesis and failure of differentiation.

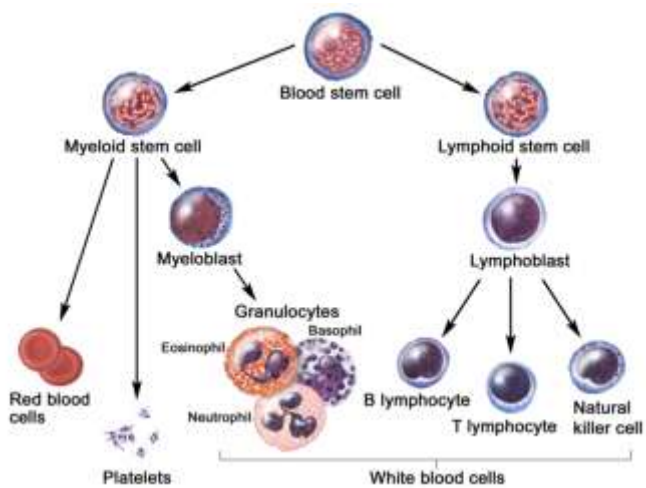

Figure 1. Blood cell development. PDQ Cancer information, National Cancer Institute US, 2002.

Classification of leukemia

Leukemias can be classified into acute or chronic leukemias depending on the untreated course and speed of presentation (Figure 2). Acute leukemias usually present as hemorrhage and bruising, anemia, infection, or infiltration of organs rapidly and patients often present unwell. However, many patients with chronic leukemias are asymptomatic but can have splenomegaly, fever, weight loss, malaise, frequent infections, bleeding, thrombosis, or lymphadenopathy. Some chronic leukemias enter an acute blast phase where the clinical manifestations are similar to the acute leukemias and can be hard to differentiate.

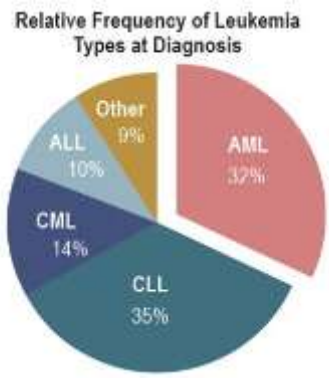

Figure 2. Subtypes of Leukaemia. Cancer Facts & Figures, American Cancer Society, 2018

Morphologically, under the microscope, leukemia cells can look very similar (large, dense nuclei and little cytoplasm increasing the nuclei-cytoplasmic ratio) so expressing antigens found also on blasts cells and can help to further differentiate between them. For AML common differentiation (CD) markers include CD13, CD33 and CD34 (Campos L et al. 1989) with monocytic differentiation markers (CD4, CD14, CD11b), erythroid (CD36, CD71) and megakaryocytes markers (CD41a and CD61). Lymphoblasts both T or B cell lineages express different antigens such as Terminal deoxynucleotidyl transferase (TdT), Human leukocyte antigen-antigen D related (HLA-DR), CD7 and CD19, CD4, and CD8. Mixed phenotypic leukemia that carry a worse overall prognosis leukaemia both myeloid and lymphoblastic immunophenotypic features co express (Wolach O et al, 2015).

The important of getting the right diagnosis and subclassification is underpinned in the prognosis and long-time survival of AML. AML can be cured in only 35%–40% of patients younger than age 60 years old (Döhner H et al. 2015). For those >60 years old, the prognosis remains grim especially as tolerance to intensive therapy into this age group can be challenging. This is important as the age distributions of AML is generally a disease of older people and is uncommon before the age of 45 with a median age of 69 years. The average age of people when they are first diagnosed with AML is about 68-year-old but is can occur across all age groups and even in children. Recent studies have revealed that the disorder arises from a series of recurrent hematopoietic stem cell genetic alterations accumulated with age this is known as a clonal evolution in which there are founder mutations that give rise to subclones and then driver genes mutations with a more proliferative phenotype (Ding L et al. Nature. 2012).

Over the years there have been several different classification systems for AML adapted with the advent of new prognostic information emerging from clinical trial analysis and biomarker discoveries programs. These

tended to be based initially on the aetiology e.g de novo AML, treatment related AML, secondary AML in the case of myelodysplastic syndrome (MDS) transformation to AML. MDS are clonal disorders of myeloid stem cells. That are characterized by ineffective hematopoiesis manifested in morphologic dysplasia of hematopoietic precursors that give rise to one or more peripheral blood cytopenias. Around 30% can go on to develop AML.

The morphology and immune-phenotype of leukemia cells was the basis of the French-American-British classification system mainly defining eight major AML subtypes (FAB M0 to M7) (Bennett J.M et al, Br. J. Haematol. 1976). More recently the World Health Organization (WHO) classification and the European Leukaemia working party (ELN) are using the genetic characteristics of AML classify this disease into biological subtypes (Döhner H et al, Blood 2017). With these modern classification systems physicians can stratify patients into prognostic groups e.g favourable, intermediate and adverse and the offer appropriate risk-based therapy and avoid the potential of over and under treating of patients.

Treatment of AML for decades has involved intensive chemotherapeutic agents such as cytarabine, daunorubicin and idarubicin. After inducing and remission in the induction phase patients usually move on to consolidation with more intensive chemotherapy to combat any resistant disease and usually an allogenic bone marrow transplantation in which the entire stem cell population of the receipt bone marrow is replaced by a donor stem cells. Unfortunately, even with this approach half of the patients relapse and die of AML (Figure 3) (Klepin et al J Clin Oncol, 2014)

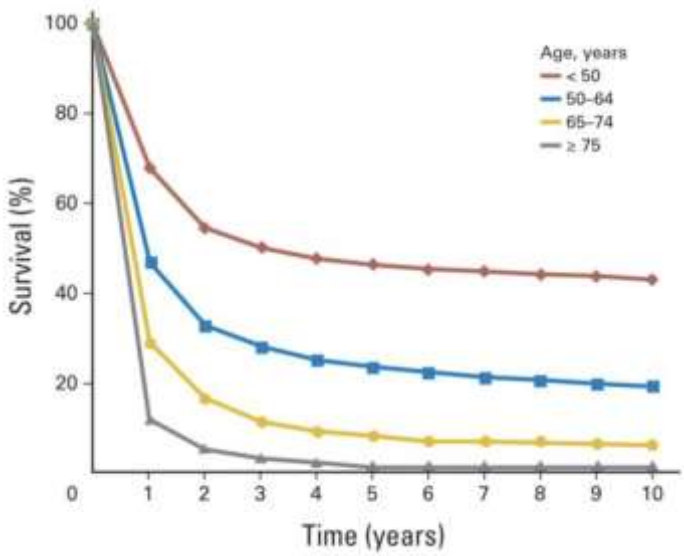

Figure 3. Survival of AML by age group. Klepin et al, J Clin Oncol, 2014.

Biology of leukemia

Understanding the molecular biology of AML is crucial to improving survival. Not only will this aid with our understanding of what the molecular drivers are for progression, resistance to chemotherapy but also pave the way for novel targeted intervention. Mutations in the FLT3 gene are one of the most frequent somatic alterations in AML, resulting in ligand-independent activation that leads to hematopoietic transformation. In a study of 1540 patients, FLT3 internal tandem duplications (ITD) were seen in 22% of patients and tyrosine kinase domain (TKD) mutations, such as D835, were seen in 8% of patients (Figure 4). (Papaemmanuil et al., 2016). In addition, other variant FLT3 mutations were seen in 5% of AML samples, suggesting that FLT3 is an important driver of leukemogenesis in a subset of AML. This is important as this has led to the development of FLT3 inhibitors and the conduction of clinical trials that have untimely resulted in approval of such agents e.g Midostaurin and Gilteritinib for FLT3 mutant AML. Other targeted agents approved include IDH1 inhibitors such as Ivosetinib. IDH1/2 mutations occur in 20% of AML and represent another class of frequently occurring molecular aberrations in AML, with IDH1 and IDH2 mutations noted in 6–16% and 8–19% of AML cases, respectively and can be acquired at time of progression (Molenaar et al, 2015, Dang L, 2010; Chou et al, Leukemia 2011).

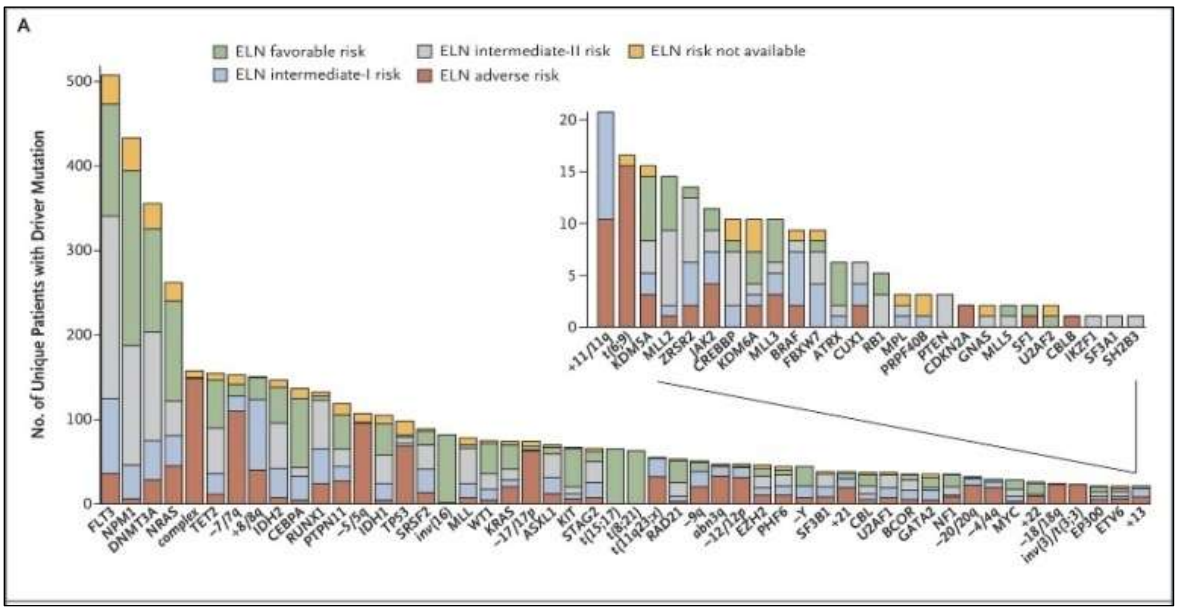

Figure 4. Frequency of mutations in 1540 AML patients (Papaemmanuil et al., 2016)

It is now standard of care to perform a bone marrow aspirate and biopsy, including morphology, immune-phenotype, cytochemistry and genetics studies (conventional karyotype and molecular studies) for essential

for diagnosis, classification and risk stratification.

Prognosis of Leukemia

Several factors such as age at diagnosis, initial white blood cell count, leukaemia subtype, gender, chromosome changes and response to initial treatment can affect the prognosis of patients with acute myeloid leukaemia (AML). These factors help govern decisions on the intensity of the treatment (Arceci R *et al*. 2016). Patients are often categorised into low, standard, high or very high risk group. Those at low risk group have a better prognosis than those at very high risk group. Intensive treatment is given to those in very high risk group. Conventional prognostic factors in AML

Age at diagnosis: The patient's age does affect the prognosis with younger people having a better prognosis than older people with 5 year survival rates now ranging from 65 to 70% compared with aged 65 or older, who have a 5% 5 years overall survival rate (Horton TM *et al*.2018).

Furthermore, children under the aged 14 tend have an even better prognosis with a 5 years of 65%. Around 60% of patients aged 15 to 24 tend to survive 5 years or more after being diagnosed. In people aged between 25 and 64, 40% survive for 5 years or more after they are diagnosed.

Initial white blood cell count: patient with WBC count less than 100,000 cells per cubic millimetre at diagnosis tend to do better than those with higher WBC count.

Karyotypic features: Some specific genetic changes in the patient's leukaemia cells may make it harder to treat effectively. For example,

Response to initial treatment: It is quite obvious that patients whose leukaemia responds quicker to chemotherapy are more likely to be cured then those whose leukaemia takes longer to respond or doesn't respond at all.

Changing from chronic to acute: The outcome of the treatment highly depends on the on whether the patient has had a leukaemia that has transformed from a chronic into acute form. This is because, it can be difficult to treat leukaemia that has transformed or has developed from myelodysplasia.

Secondary leukaemia: Secondary leukaemia is a leukaemia that develops after treatment for another cancer. This is a rare condition caused by damaged to the bone marrow by chemotherapy. When this occurs leukaemia may become more difficult to treat successfully.

It is common for patient with MRD to have a relapse. For patients to have relapse, more than 5% of their bone marrow must be made up of leukaemia cells or blast cells.

Some types of leukaemia are inherited through germline mutations. Familial predisposition to leukaemia has been a topic of interest for decades. For many years, a connection between inherited forms of myelodysplastic syndrome (MDS) and myeloid leukaemia were established in several disorders in childhood such as Fanconi

anaemia (Jakub et al, 2019). This has prompted more research to be conducted which led to the discovery of a number of additional inherited bone marrow conditions that cause leukaemia. High clinical penetrance for haematological disorders in these patients and the repeated occurrence of the similar phenotypes in family members have enabled the identification of the respective genetic causes for these conditions. These conditions are known to be caused by germ line mutations in the genes involved in the development and maintenance of haematopoietic system and are referred to as Familial Acute Myeloid Leukaemia (FAML).

FAML is inherited in a variety of genetic patterns including autosomal dominant, Autosomal recessive patterns. It is characterised by a large number of abnormal immature leukocytes known as myeloid blasts. These Myeloid blasts then develops into malignant leukaemia cells which interfere with the production of functional leukocytes, erythrocytes and platelets. FAML is very rare, only a few affected families have been identified. It often begins earlier in life and has been reported in children and even infants.

With the advent of high through put and deep sequencing such as Next Generation Sequencing (NGS) technologies has enabled the identification of many genes associated with hereditary leukaemia's such that it is now possible for screening interventions to take place in asymptomatic individuals as well as strategies to be adopted for optimal therapeutics.

Summary

Significant progress has been made to understand the molecular biology of AML in order o correctly classify patients into risk-based groups and allow for therapeutic decisions. Much of this has been as a result of high throughout technologies such a as next generation sequencing in which new biomarkers have been uncovered. In addition, it has helped us uncover why a proportion of patients remain resistance to standard therapy or develop more significant toxicity that expected such as bone marrow failure. The work presented in the dissertation will focus on familial myeloid leukemia, our understanding of the molecular events that give rise to their clinical phenotypes and what lessons have been learnt from these rare subtypes of leukemias that can be applicable to all leukaemia patients.

Current treatment approaches to leukaemia

Acute Myeloid leukaemia represents a clonally diverse disorders with both biological and clinical heterogeneity. The clonal evolution of these disorders has uncovered different avenues to investigate and develop targeted therapies towards however due to the diverse genomic and co-occurring mutations the main stay of therapy for the last 40 years has been cytotoxic chemotherapy based. Chemotherapy is the most common treatment for people with leukaemia. The fact that chemotherapy drugs are administered into the blood stream and reach all areas of the body makes it ideal for cancers such as leukaemia. Chemotherapy

agents in AML are used to target fast proliferating cells undergoing rapid divisions through the cell cycle, especially the 's' phase in which DNA synthesis occurs. Many agents used for AML therefore specifically have their mode of action by targeting DNA synthesis and stability.

Standard of care chemotherapy

The purpose of treating patients with AML is to induce a remission defined as having no evidence of leukaemia cells after the initial treatment. This is characterised by: the bone marrow containing less than 5% of blast cells, blood cell counts being within the normal limits and no sign or symptoms of the disease being present (Tarlock K *et at*. 2018). Long term remissions require eradicating resistant and persistent leukaemic clones that are not easily detectable using standard methods. Measurable residual disease (MRD) is where leukaemia cells cannot be found in the bone marrow using standard lab tests such as morphology but they can still be detected with more sensitive tests such as flow cytometry. It is common for patient with MRD positivity to relapse.

The standard treatment for AML is given in two phases:

Induction phase: this is short and intensive and often lasts about 4 weeks with the purpose to clear leukaemia cells in the blood and to reduce the number of leukaemia cells in the bone marrow. This phase involves the combination of Cytarabine (Cytosar-U) and Anthracycline drug such as daunorubicin (cerubidine) or idarubicin (idamycin). The schedule of tis has enable its name '7+3' to be adopted worldwide. Cytarabine is given as a continue 7 day infusion of 100mg/m2-200mg/m2 and anthracyclines as short infusion on D1,2, and 3. Patient need to stay in hospital during induction phase for 3 to 5 weeks for their white blood cell counts to return to normal (Kebriaei P et al. 2015). At the end of this phase a reassessment bone marrow aspirate is done to review whether patients have responded to this treatment and cleared blast cells in the bone marrow to <5% (remission) or whether there remains refractory disease indicative of resistant cells in which treatment escalation is required

Consolidation phase: chemotherapy is given after the patient has recovered from induction phase, with the purpose to kill the remaining leukaemia cells that are still present in small number but can't be seen. This is called 'measurable residual disease' (MRD). For this phase, chemotherapy is given in cycles and each cycle followed by a rest period to allows the body to recover. Younger patients are often given 2 to 4 rounds of high or intermediate dose of Cytarabine at monthly intervals 3g/m2/dose. Several different regimens are used for older patients and even nowadays non-chemotherapy based options such as Venetoclax (BCL2 inhibitor) and Azacitidine (hypomethylation agent). A bone marrow /stem cell transplantation is often recommended as

consolidation therapy for younger patients whom molecular and cytogenetic studies predict a poorer prognosis (Kebriaei P *et al*. 2015).

Maintenance phase: Due to reports identifying the risk of relapse after consolidation, many studies have now explored possibly maintenance therapy in this setting in at attempt to improve the relapsed free survival post consolidation. These agents include FLT3 inhibitors such as midostaruin and sorafenib in FLT3 mutated AML and azacytidine in older patients (Schlenk et all Blood 2019). Randomised trials are still underway evaluating this further. The challenge with these is to develop an MRD platform in order to prove that the impact of a maintenance agent appropriately clears the desired clones and offers deep and durable remissions.

Chemotherapy drugs can have side effects depending on the type and dose of the drug and how long they are taken. They attack rapidly dividing cells including those in healthy tissues such as hair, lining of the mouth and bone marrow. This explains why people who receive chemotherapy lose their hair. Low white blood cell count is the most common and dangerous side effect as this renders the patients at high risks of contracting life threatening infections. Drugs such known as growth factor such as neupogen, neulasta and leukine are sometimes given to patients to help increase white blood cell count and lower the risks of infection. Low platelet counts and low red blood cell counts can are also among the side effects and can be treated by platelet transfusion and red blood cell transfusion. Chemotherapy can also damage organs such the kidneys, liver, testicles, ovaries and lungs (Anthony Nolan. 2016).

Additionally, Cytarabine is neurotoxic and can cause confusion, ataxia and seizures. Rarely can it cause (posterior reversible encephalopathy syndrome). Anthracyclines drugs such as daunorubicin and idarubicin are cardiotoxic and therefore pro dose echocardiograms and ECG's are often don't to identify and underlying cardiac issues before delivery. Dose reductions and capping of the total dose of anthracycline is therefore common practice in leukaemia management.

Tumour lysis syndrome tends to occur during the induction phase of the treatment. This is caused when leukaemia there is rapid cells break open and release their intracellular contents into the blood stream such as potassium and calcium, as a result this syndrome can result in grossly altered biochemistry, renal impairment, fluid retention and even death. (Larson RA *et al*. 2018).

The role of bone marrow transplantation

Bone marrow or stem cell transplant is a procedure that involves the destruction of bone marrow containing leukaemia and replacement with specialised haematopoietic cells that develop, into healthy bone marrow. Prior to being recommended for this procedure, the doctor will discuss the risks involved with the patient. The purpose of this procedure is to destroy all the cancer cells in the marrow, blood and other parts of the body

using high doses of chemotherapy and/or radiation and allow replacement blood stem cells to create healthy bone marrow. There are two types of stem cell transplantation depending of the source of the replacement blood stem cells:

Allogeneic (ALLO), involves high dose chemotherapy with donated stem cells. It's important to find a door whose bone marrow matches the patient so that human leukocyte antigen (HLA) of the donor matches that of the patient to prevent graft versus host disease (GVHD), where healthy cells from the donor attack the patient's cells. In most cases the patient's close relative such as brother or sister may be the best match to provide stem cells (Larson RA *et al*. 2018).

In contrast to ALLOs, autologous (AUTO) which also involves high dose of chemotherapy with patient's own stem cells being returned. This is infrequently done in the setting of haematological malignancies for fear of repopulating the donor marrow with leukaemic clones (Larson RA *et al*. 2018).

AML is currently the most common indications for hematopoietic stem cell transplantation (HSCT) worldwide. The rationale for the indication of HSCT is based on the fact that this disease is the AML cells have the capable of self-renewing and exhibit resistance to standard dose cytotoxic chemotherapy resulting in disease relapse. The benefits of allogeneic HSCT derive from the destruction of these stem cells by the donor's immune system [T and natural killer (NK) lymphocytes] and the graft-versus-leukemia effect.

Allogenic bone marrow transplantation, however, carries significant risks and just like chemotherapy causes significant organ toxicities, risk of life-threatening infections death. The presence of comorbidities greatly compromises the results of HSCT, as a result of which this therapeutic option is not recommended for patients with high comorbidity burden. Therefore, for the elderly this is not a good treatment option. Likewise, for younger more favourable patients, allogenic BMT may not be required at all. A recently published meta-analysis, which included 6700 patients enrolled in prospective trials on HSCT for AML in first remission showed Allogeneic HSCT is not indicated for low risk patients[(23)] especially those with core binding factor (CBF) leukaemia, CBF-AML [(8;21), inv(16) or t(16;16)] or acute promyelocytic leukaemia [t(15-17)] have a moderate risk of relapse and around 50% survival in 5 years when they undergo only chemotherapy (Marcucci G et al, J Clin Oncol. 2005).

Targeted inhibitors and personalized in AML

The understanding of the molecular mechanism of AML has resulted in the development of targeted inhibitors either in addition to or instead of chemotherapy.

Targeting FLT3 mutations

Mutations in the FLT3 gene are one of the most frequent somatic alterations in AML, resulting in ligand-

independent activation that leads to hematopoietic transformation. In a study of 1540 patients, FLT3 internal tandem duplications (ITD) were seen in 22% of patients and tyrosine kinase domain (TKD) mutations, such as D835, were seen in 8% of patients (**Error! Reference source not found.**). (Papaemmanuil et al., 2016)

In addition, other variant FLT3 mutations were seen in 5% of AML samples, suggesting that FLT3 is an important driver of leukemogenesis in a subset of AML. The mutational profile of AML changes with age. Presence of FLT3 mutations peaks between age 45-60 years and FLT3 frequency decreases with older age thus providing a biological rationale for designing separate AML trials for patients older than 60 years. (Bullinger et al., 2017)

Midostaurin was recently approved for the treatment of adult patients with newly diagnosed FLT3-mutated AML. Midostaurin was evaluated in a double-blind placebo-controlled randomized trial in younger (≤ 60 years) newly diagnosed AML patients with FLT3 mutations (CALGB 10603, RATIFY trial). The study combined standard cytarabine and daunorubicin (7+3) induction as described previously in with fourteen days of midostaurin (days 8-21) or with placebo. Patients also received fourteen days of midostaurin or placebo after high dose cytarabine (HiDAC) consolidation.

In this 717-patient study, efficacy was established based on overall survival (OS), measured from the date of randomization until death by any cause. Midostaurin plus standard chemotherapy was superior to placebo plus standard chemotherapy in OS (HR 0.77; 95% CI 0.63, 0.95; p=0.016) (Figure 1). Because survival curves plateaued before reaching the median, median survival could not be reliably estimated (Stone et al., 2017).

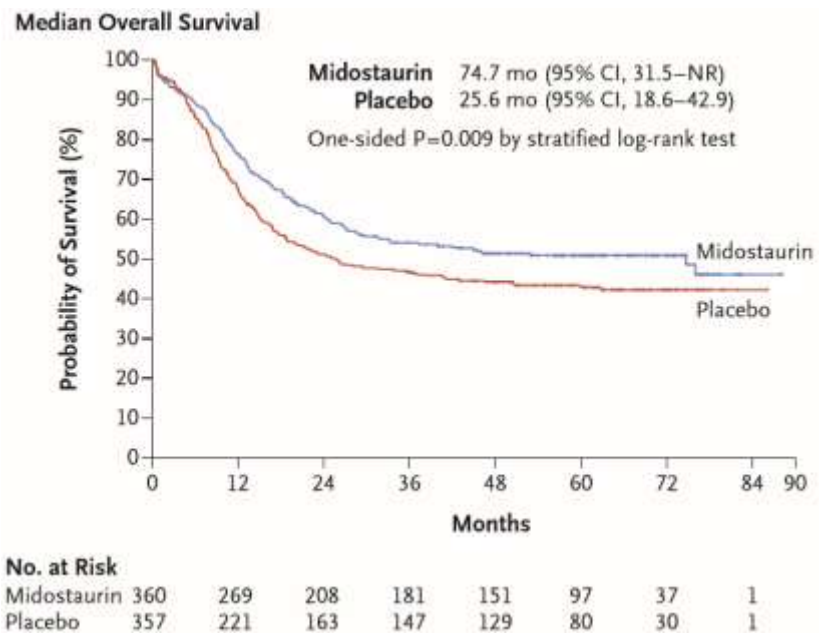

Figure 1 Overall survival of midostaurin versus placebo in the RATIFY trial (Stone et al., 2017).

The analysis of event-free survival (EFS), defined as a failure to obtain a complete remission (CR) within 60 days of initiation of protocol therapy, relapse, or death from any cause, showed a statistically significant improvement with a median of 8.2 months for midostaurin plus standard chemotherapy versus 3.0 months for placebo plus standard chemotherapy with HR 0.78 (95% CI 0.66, 0.93) and 1-sided p=0.002. The overall survival benefit at four years (7.1% improvement; 51.4% on midostaurin vs. 44.3% on placebo) was similar to the improvement in EFS (7.6% improvement; 28.2% on midostaurin vs. 20.6% on placebo)(Stone et al., 2017).

While midostaurin represents an advance in the treatment of newly diagnosed patients with FLT3-mutated AML, numerous challenges remain. Even in patients who achieved an early remission after induction even in those who received midostaurin still had a high risk of disease recurrence. The proportion of these 212 patients who remained alive and disease free at two years after staring treatment only around 50%.

In Ratify trial, midostaurin has improved the overall survival by a hazard ratio of 0.77, resulting in the regulatory approval of the drug (Rydapt) and a new standard of care for newly diagnosed AML patients with FLT3 mutation. However, the 4 year disease free survival (DFS) rate in patients who achieved CR after midostaurin was 46.4%, suggesting an ongoing risk of relapse, especially during the first year (**Error! Reference source not found.**). These data indicate that the risk of relapse remains high and there remain rooms for improvement for this patient population.

Targeting Bcl-2

Evasion of apoptosis is a hallmark of malignant tumor progression, allowing for tumor survival and resistance to cancer treatments. 1 The interaction between the BCL2 family of proteins is critical towards the regulation of the intrinsic apoptotic pathway. BCL2 is overexpressed in AML and associated with resistance to chemotherapy and poor outcomes. 2 Aberrant BCL-2 expression is essential for maintaining quiescent leukemic stem cells and BCL-2 inhibition induces cell death in leukaemia cells Figure 3.

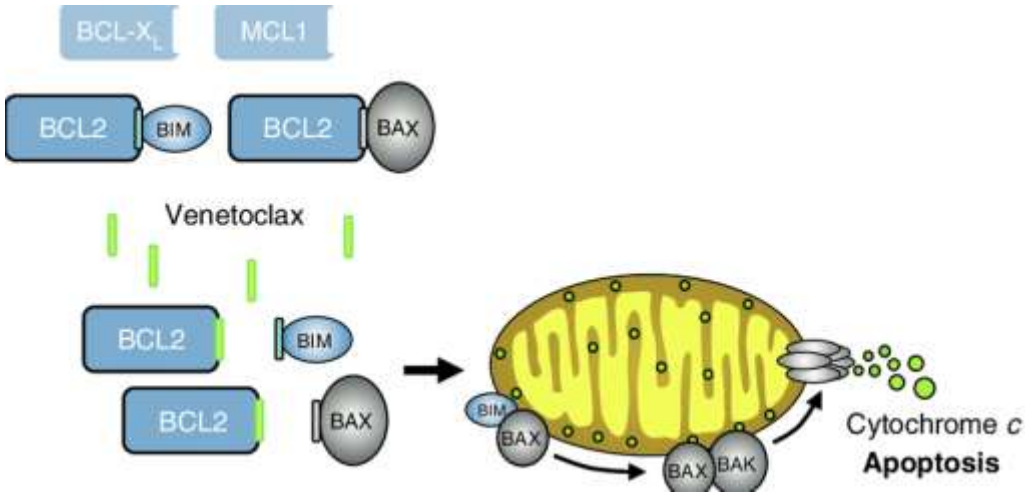

Figure 3. Mechanism of action of Venetoclax (Konaplava et al, Cancer discovery 2016)

The development of ABT-199 or Venetoclax, a potent and selective inhibitor of Bcl-2 is a remarkable advancement in the field of AML. In a phase II study of Venetoclax (800mg daily) in patients with high-risk relapsed/refractory AML or unfit for chemotherapy the ORR was 19%, CR 6%, and median progression free survival was 2.5 months. 4 In order to improve these outcomes, recent studies have evaluated combinations of Venetoclax with other therapies active in leukemia. Preclinical in vitro and ex-vivo studies demonstrated that Venetoclax synergizes with the hypomethylating agent, azacytidine. 5 This led to a phase 1b study of Venetoclax with decitabine (20mg/m2 days 1-5 of a 28-day cycle) or azacytidine (75mg/m2 days 1-7 of a 28 day cycle), which showed a complete remission or complete remission with incomplete marrow recovery (CR/CRi) in 61% and median OS of 12.3 months in elderly patients with previously untreated AML. The maximum tolerated dose of Venetoclax was not reached and the phase 2 recommended dose was 400mg daily or 800mg intermittently. Overall the combination was well tolerated with a low early mortality rate of 7%. 6 As a result, Venetoclax was granted breakthrough designation by the FDA, and phase III studies are ongoing. Given these remarkable response rates, Venetoclax in combination with hypomethylating agents will likely become the standard of care first line therapy for newly diagnosed patients unfit for chemotherapy. In order to further improve outcomes, future trials will need to evaluate novel combinations of therapies that include a backbone of Venetoclax and a hypomethylating agent. Targeted therapies in AML including FLT3, IDH and JAK2 inhibitors are promising agents for combination with Venetoclax and azacytidine, since they are highly effective as monotherapy and with the exception of ruxolitinib, have minimal overlapping toxicities.

Targeting IDH1/IDH2 mutations

Ivosidenib is an isocitrate dehydrogenase-1 (IDH1) inhibitor now FDA approved for the treatment of adult patients who have relapsed AML and a susceptible IDH1 mutation. (Dinardo CD et al., NEJM 2018) Enasidenib is an IDH2 inhibitor now FDA approved for the treatment of adult patients with relapsed AML and a susceptible IDH1 mutation (Stein EM et al., Blood 2017) Ivosidenib and enasidenib have been combined with standard AML induction therapy in a phase 1b study of 80 patients (ivosidenib + Chemotherapy n=30, enasidenib + Chemotherapy n=50). The combination of ivosidenib or enasidenib with standard AML induction therapy was found to be safe and well tolerated. Some patients with secondary AML (sAML) treated with enasidenib exhibited a prolonged time to platelet count recovery, potentially reflecting reduced hematopoietic reserve of patients with sAML. Preliminary efficacy data are consistent with expectations for 7+3 induction, but long-term follow-up data are awaited. A randomized phase III trial is planned to assess the efficacy of ivosidenib or enasidenib combined with standard induction therapy in patients with newly diagnosed AML with IDH mutations (Stein EM et al., ASH 2017)

Given their distinct mechanisms of action, targeted therapies have the potential to lead to higher response rates, deeper responses with greater percentage of MRD negativity and more durable responses when used in combination with chemotherapy and shifting to a new paradigm doe the treatment of AML.

Summary

There is now the variability of novel targeted inhibiters which add to the conventional approaches to treating patients with AML fundamentality to improve survival in patients, especially those in poorer risk groups. Over the next few years it will be interesting to see whether chemotherapy and allogenic transplantation will have a much smaller role in the front-line approach as subsets of patients with more biologically favourable groups are teased out of the clinical trial data that emerges.

Non syndromic and Syndromic familial Leukaemia

Familial Acute Myeloid Leukaemia (FAML) arising in the context of patients with inherited mutations is becoming mor apparent with the advent of new technologies. These mutations are not known to be associated with any syndrome and this chapter summaries the common germline mutations, pathophysiology and implications for management. Furthermore, an increasing number of inherited AML predisposition syndromes have been recognized that have additional phenotypic findings and often present in childhood. This chapter summaries these diverse biological and clinical disorders of syndromic and non-syndromic AML.

Non syndromic FAML

Familial AML with mutated CEBPA

Familial AML with mutated CEBPA is inherited in an autosomal dominant fashion and displays complete or near-complete penetrance for development of AM and generally of favorable prognosis (Owen *et* al. 2008). Different to CEBPA mutant sporadic AM the familial form is associated with biallelic CEBPA mutations, most commonly within the 5' end of the gene, accompanied by acquisition of a second 3' mutation in the leukemia.

CEBPA encodes a master hematopoietic transcription factor that acts as a critical regulator of granulocyte and monocyte differentiation. This produces a shorter version of CCAAT enhancer- binding protein alpha. This shorter version is produced from one copy of the CEBPA gene in each cell and is believed to interfere with the tumour suppressor function of the normal protein produced from the second copy of the gene. The absence of the tumour suppressor function is believed to disrupt the regulation of blood cell production in the bone marrow, via gene repression occurring as a consequence of promoter methylation and the action of leukemia-specific translocation fusion proteins, leading to the production of abnormal cells that are seen in AML

(Tawana K *et al*. 2010).

In addition to the inherited mutation in one copy of the CEBPA gene in each cell, most individuals with FAML with mutated CEBPA also acquire a mutation in the second copy of the CEBPA gene which is called somatic mutation. This is known as biallelic CEBPA FAML. This mutation is only present in the leukaemia cell and is not inherited. However, the effect of this second mutation on the development of AML is still unclear but thought to be as a result of a decrease the DNA-binding ability of CCAAT enhancer-binding protein alpha (Carmichael CL *et al*. 2010).

Clinically, germline CEBPA mutations confer no specific genotype–phenotype relationships, with the familial and sporadic forms share similar pathologic features, including normal cytogenetic analysis, a predominance of FAB subtypes M1 and M2. There are no dysmorphological features. Given that the prognosis is relatively favourable the management of these patients heading for bone marrow transplantation needs to be carefully considered and the risk -benefit of this assessed.

Familial MDS/AML with mutated GATA2

Germline testing has identified and linked GATA2 mutations in families who had developed MDS/AML. Individuals who carry GATA2 mutation have been reported to display no distinctive phenotypic abnormalities other than a highly penetrant autosomal dominant inheritance of early-onset myelodysplastic syndrome (MDS) and AML (Hahn *et* al. 2011). GATA2 gene encodes a member of the GATA family of Zinc-finger transcription factors that bind to the promoter regions of the target genes to initiate the transcription of a protein involved in the regulation of transcription of genes essential in the development and proliferation of hematopoietic and endocrine cell lineages. GATA2 mutations are linked with a variety of inherited and acquired immune disorders, MDS and AML (Ishida *et* al. 2012).

Clinical challenges in this relatively young population include often the poorer response to standard chemotherapy and need for escalation of treatment with a bone marrow transplant. Given this highly penetrance nature the need to look for donors outside the family is critical.

Familial platelet disorder with myeloid malignancy (FPD)

FPD/AML is a rare disorder characterised by clinical bleeding caused by platelet dysfunction and a tendency to develop myeloid malignancies. Among the families diagnosed with this condition, the majority of those who harbour the malignancy have hemizygous mutation in the RUNX1 gene. This gene encodes one subunit of a heterodimeric transcription factor that control genes essential for haematopoiesis (Churpek *et* al, 2010).

RUNX1 was first identified as one of the genes implicated in pathogenic translocation found in acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL). It encodes the alpha subunit of the core binding

transcription factor (CBFa), a heterodimeric transcription factor which regulates the differentiation of haematopoietic cells. RUNX1 promote differentiation of haematopoietic cells by interacts with core binding factor beta (CBFb) and targets promoter regions through the conserved Runt homology domain (RHD), therefore regulating transcription of multiple genes involved in normal hematopoietic development. (Rong He (Hematopathology-Third Edition, 2018).

RUNX1 is also a known to possess tumour –suppressor properties, and mutations in this gene often results in loss of function of the gene which then contributes to tumorigenesis in myeloid cancers. Most mutations occurring throughout this gene seem to have deactivating effect on its function either by disrupting the C-terminal protein interaction domain or affecting the DNA-binding RUNT domain (Eric M *et* al, 2013).

The fact that different FPD/AML families have varying risks of progressing to myeloid malignancy reflects the fact that each family carries unique mutations that disrupt various domains within the protein, therefore there is phenotypic heterogeneity. Clinically, in addition to thrombocytopenia other clinical characteristics are observed such as eczema, psoriasis, psoriatic arthritis, rheumatoid arthritis, and juvenile arthritis. Carriers of the same RUNX1 mutated gene display heterogeneity even in their degree of platelet dysfunction and thrombocytopenia, with some being asymptomatic and other having clinically significant bleeding history such as prolong epistaxis, extensive bruising and menorrhagia. The cancers developed in RUNX mutant individuals also vary including breast, prostate, lymphoma as examples (Brown A et al 2020). This analysis did not reveal a significant correlation between the type or location of mutations and malignancy. There was also a lack of correlation between the subtype of haematological malignancy and type of ***RUNX1***mutation. Figure 1. (Brown et al 2020).

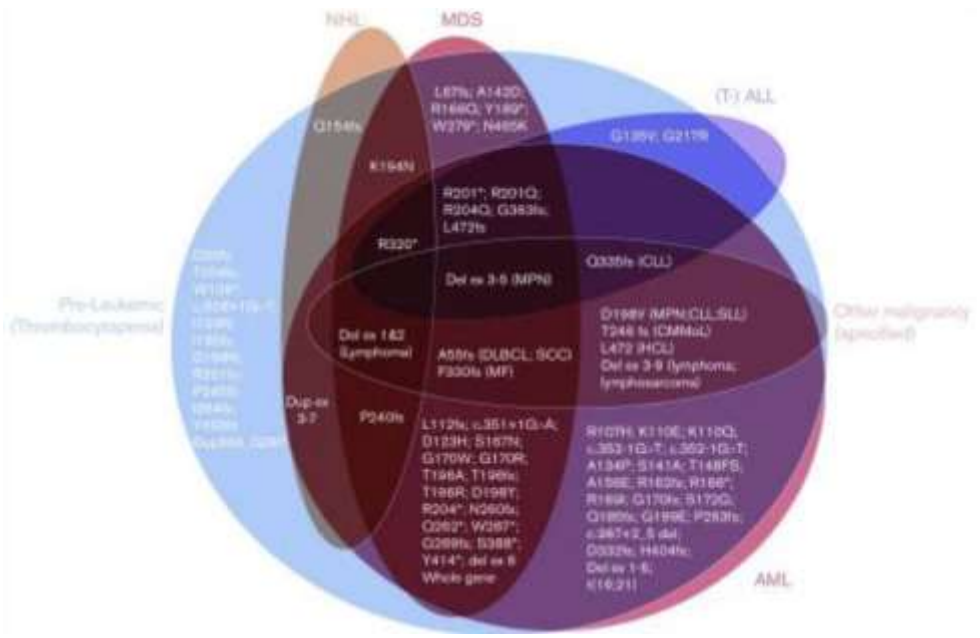

Figure 1. The spectrum of hematological phenotypes reported with different germline *RUNX1* mutations

(Brown et al 2020).

Autosomal dominant chronic lymphocytic Leukemias

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia. The cause of CLL remains unknown and family history of CLL is the best characterized risk factor. The incidence of CLL increases from the median age of diagnosis from 35 to 65 years. Families with autosomal dominant inheritance of CLL are very common in literatures, and it has been reported that 15 – 20 percent of patients have history of a lymphoproliferative disorder (LPD) (Bowen et al. 2008). There is also an established relationship between HLA and Hodgkin's lymphoma and the association between autoimmune disease and CLL increases the possibility that genes within the MHC region may be responsible for CLL susceptibility (Klitz W et al. 1994). Evidence from epidemiological studies and family studies suggests that a subset of CLL may also be caused by an inherited predisposition which results in the familial form of CLL. Identification of genes underlying familial forms of CLL may be useful for the diagnosis and treatment of those conditions as well as serve as model for tumorigenesis of these conditions in general (Houlston RS et al. 2020).

Research has been devoted to explaining the mechanisms underlying the genetic risks of this condition. Familial CLL appears to be clinically and biologically similar to sporadic CLL, where most of it tends to be preceded by monoclonal B-cell lymphocytosis (MBL). So far neither linkage or candidate gene association studies have been able to pinpoint the cause of the inherited CLL. However, genome-wide association studies (GWAS) have managed to identify multiple low-risk variants that together explain 16 percent of familial risk of CLL. Studies of individual families have identified higher risk single nucleotide polymorphism or copy number variants associated with disease risk in those families. Current studies are focusing on next generation sequencing to identify additional risk loci to the germline of CLL families and families with sporadic CLL (Goldin et al. 2010).

Autosomal recessive childhood MDS with monosomy 7

Familial monosomy 7 is characterized by early childhood onset of bone marrow failure which is often associated with increased risk of MDS and AML. The bone marrow failure/MDS/AML is usually preceded by the identification of blood leukocytes with monosomy 7. Almost all individuals reported with this condition have died of their disease. In all reported individuals, Monosomy 7 is believed to be caused by deletion of the long arm of chromosome 7 (7q-), because the deletion of the long arm of chromosome 7 is frequent cytogenetic finding is in the bone marrow of patient with MDS and AML (Shannon et al. 1989). The consistent absence of cases pedigree mapping in other generations in reported families is indicative of autosomal recessive inheritance.

For many years monosomy 7 (−7) and interstitial deletions of chromosome 7 (del(7q)) have been established as one of the most frequent chromosomal aberrations found in essentially all types of myeloid tumors regardless of patient age and disease etiology. Ongoing research has attempted to identify a recessive myeloid tumor-suppressor genes by looking at commonly deleted regions (CDRs) in del(7q) patients. However, these efforts have not yet been successful. Using powerful new technologies such as microarray comparative genomic hybridization and high-throughput sequencing allow comprehensive searches throughout the genes encoded on 7q. Among those proposed as promising candidates include *SAMD9* and *SAMD9L* mutations of which cause hereditary diseases with strong propensity to infantile MDS harboring monosomy 7.

Preclinical work has identified that MDS develops in *SAMD9L*-deficient mice over their lifetime, *SAMD9*/*SAMD9L* are likely responsible for sporadic MDS with −7/del(7q) as the sole anomaly. Additionally, the loss of *EZH2* and/or *MLL3* (both well known to be adverse biological biomarkers in sporadic AML) disturbs the epigenetic control of these already-abnormal or even already-leukemic cells and therefore potentially promote disease development (Inaba T et al 2018).

Syndromic FAML

Important DNA repair syndromes

Autosomal recessive syndromes of DNA repair deficiency are well known for causing predisposition to haematological malignancies and represent a heterogenous group of disorders from both the biological and clinical perspectives.

Fanconi syndrome

Fanconi anaemia (FA) is an autosomal recessive inherited bone marrow failure syndrome (IBMF) associated with growth retardation, organ malformation and predisposition to AML.

Clinically these patients have physical abnormalities such as short stature, microcephaly, developmental delay, café-au-lait skin lesions, and skeletal abnormalities. Diagnosis is usually made in childhood, although diagnostic delays and variable disease manifestations are common, and some individuals may not be diagnosed with FA until adulthood. At least 12 different variations of the FANC gene has been \identified in this condition with the caused by deletions, frameshifts, stop codons, splice-site mutations and missense mutations. FA is associated with the development of anomaly myelopoiesis and it is estimated that at least 20% of patient with FA develop AML by the age of 40 (Kutler *et al*.2003). Most of the genes associated with FA play a major function in repairing DNA crosslinks associated with FA/BRCA pathway (Wang, 2007).

Since FA causes hypersensitivity to DNA crosslinking agents, Diepoxybutane (DEB), a DNA crosslinking agent is commonly used in the DEB –induced chromosome breakage assay to diagnose FS. Peripheral blood

from a patient suspected with FA is cultured and treated with DEB, which induces irreversible DNA crosslinking and chromosome breakage that is visible in Giemsa-stained metaphase cells. This is often referred to as the DNA fragility test.

Haematopoietic stem cell transplantation (HSCT) offers the only cure for hematopoietic abnormalities associated with FA (Gluckman *et al*.1995). It is therefore important to confirm the presence of FA or exclude it when evaluating siblings as HCT donors, so that the patient does not receive hematopoietic stem cells from a sibling with FA.

Bloom syndrome

Bloom syndrome (BS) is an inherited disorder characterized by short stature and a skin rash that develops after exposure to the sun and causes an increased risk of cancer. Affected individuals tend to develop learning disabilities, increased risk of diabetes, chronic obstructive pulmonary disease (COPD) and mild immune abnormalities that leads to recurrent infections of the upper respiratory tract, ears and lungs during infancy (Singh DK *et al*. 2009).

BS is inherited in autosomal recessive and is rare disorder and so far only a few hundred affected individuals have been reported in medical literature and is caused by mutations in BLM gene. The BLM gene encodes a member of protein family known as RecQ helicases. Helicases are also known as "caretakers of the genome" because they help to maintain the structure and integrity of DNA (Liu Y, 2008). Mutations in BLM gene result in the absence of functional BLM protein. As a result, increased frequency of sister chromatid exchanges and chromosome breakage occurs more frequently; and the cell is less likely to repair DNA damage. AML, ALL, lymphoma and other malignancies occur in about 25% of patients. (German J *et al*. 2005).

Ataxia Telangiectasia

Ataxia telangiectasia (AT) is a genetic neurodegenerative disorder that becomes prominent early in childhood. It is characterized by progressive impaired coordination of voluntary movement (ataxia), the development of reddish lesions on the skin and mucous membranes caused by permanent widening of groups of blood vessels (telangiectasia) and impaired function of the immune system. Because of impaired immune response, affected individuals may be susceptible to chronic lung infections, chronic pneumonia and chronic bronchitis. Individuals with AT also have high risk of developing malignancies such as lymphoma, leukaemia (Gatti RA, 2003).

AT is inherited as an autosomal recessive trait and is caused by mutations in ATM gene, located in the long arm (q) of chromosome 11. ATM protein made by ATM gene is an enzyme that that responds to DNA damage by triggering the accumulation of the p53 protein. In individuals with AT mutations in the ATM gene result

in the absence or the defect of the ATM protein which cause delayed accumulation of the p53 protein. As a result, cells with DNA damage continue to replicate increasing the risk of cancer development (Savitsky K *et al.* 1997). Similar to Fanconi a Anemia and Blooms syndrome haematopoietic stem cell transplantation (HSCT) offers the only cure for hematopoietic abnormalities associated with FA

Constitutional trisomy syndromes

Down's syndrome

Down's syndrome (DS) is characterised by dysmorphism, mental retardation with hypotonia, gastrointestinal anomalies and frequent hereditary heart disease. Individuals with Down syndrome have up to ten fold increased risk of developing leukaemia. Acute myeloid leukaemia tends to develop before the age of 3 years of age, with FABM7 megakaryoblast being the predominant subtype (Rosner F et al. 1972). DS is caused by Trisomy 21 and less commonly mosaicism (Antonarakis SE et al.2004).

DS fetal livers show enhanced erythroid and megakaryocytic and so Trisomy 21 is considered to be the first genetic event leukemogenesis, the second hit is a mutation of the X-linked gene ***GATA1***, a transcription factor essential for development of the erythroid and megakaryocytic lineages. ***GATA1*** mutations are associated with the development of abnormal myelopoiesis and a pre leukemic condition known as transient abnormal myelopoiesis (TMD) in DS babies. This confirms the clonal nature of AML and its evolution from TMD.

Summary

Insights have been gained through the genomic profiling of patients with family history of haematological disorders and cancer with the identification of familial non-syndromic AML entities and syndromic AML. As they are biologically and clinical diverse, they have helped significantly in our understanding of the clonal nature of AML in these patients and pave the way for best practices in management.

Translational investigations in familial acute myeloid leukaemia

Germline testing for familial cases of myeloid leukemia in adults is becoming more common now that there is the recognition of these multiple genetic syndromes predisposing people to bone marrow disease. Clinical Laboratories have now approved testing platforms for several myeloid leukemia predisposition syndromes that are now included in the routine diagnostic work up of an acute leukemia patient. What remains challenging is how this information is used to direct changes in the care of patients and improve the outcome of patients with FAML taking into account many ethical considerations.

Sampling for the identification of FAML

The method of obtaining germline DNA is of paramount importance for patients with hematologic malignancies. While it is easy to obtain a germline DNA sample, e.g. peripheral blood DNA, from patients with solid tumours, it is very challenging to obtain germline DNA from patients with haematological malignancies. Blood is not acceptable (and often not available pre diagnosis) due to the inability to distinguish germline from somatic mutations, and saliva is not recommended due to frequent tumor contamination (Heinrichs S et al, Blood. 2010).

Skin fibroblasts are the most appropriate germline controls with a 4mm punch biopsies from patients is usually for sufficient fibroblast culture growth and DNA procurement. It does, however, take 6–8 weeks from the time of procedure to obtaining results.

Other sources such as buccal smears or sputum samples have a high potential to be contaminated with malignant blood cells. Less commonly used source for germline DNA are finger or toenails. In one study this was evaluate further by the identification of somatic mutations in AML using fingernail DNA as germline reference. The authors extracted DNA from fingernail clippings of AML patients and successfully used this DNA to unambiguously identify somatic mutations in the DNA extracted from a leukemic BM sample of the same patient at the time of diagnosis (Kakadia et al, Scientific reports, nature commun 2018).

Molecular assays

The detection of genetic alterations using molecular methods is preferably the gold standard for diagnosis. The anticipated rapid incorporation of next-generation sequencing (NGS) into clinical practice has changed the diagnostic approach, with full exome, transcriptome, and genome sequencing becoming standard practice.

Next generation sequencing and myeloid panels

Next generation sequencing (NGS) is a massively parallel sequencing technology that allows for rapid, precise and cost-effective sequencing of multiple genes within a single day and has revolutionized genomic research. This has led to changes in the classification, prognostic stratification, treatment and response assessment of AML. Myeloid panels of genes frequently mutated in somatic MDS/AML are communing used in the diagnostic workup of newly diagnosed patients and can range from 30 genes to over 1000 genes. Although the specific genes analyzed on hematologic malignancy gene panels vary by platform and institution, most panels share a core set of approximately 25 genes that are frequently mutated in acute leukaemias and myeloid neoplasms. These core genes include several (i.e., *CEBPA*, *GATA2*, *RUNX1*, tumour protein 53 [*TP53*], and others) that can be mutated in the germline of the patient. This offers both an opportunity and a challenge to determine variants of potential germline origin and of clinical significance identified within panels designed

principally for somatic mutation analysis.

Current myeloid NGS gene panels include: (i) SureSeq myPanel™ NGS Custom AML (Oxford Gene Technology, Begbroke, Oxfordshire, UK); (ii) Leuko-Vantage Myeloid Neoplasm Mutation Panel (Quest Diagnostics, Madison, NJ, USA); (iii) AmpliSeq® Myeloid Sequencing Panel (Illumina); and (iv) Human Myeloid Neoplasms Panel (Qiagen, Venlo, The Netherlands) have been validated and are commercially available.

The lack of a normal control for NGS testing in patients with active hematologic malignancy prevents the ability to definitively distinguish whether a mutation is of germline or somatic origin. There is much contamination of samples with both normal and cancerous cells e.g, blood samples contain leukaemia cells as well as normal cells. In the absence of a matched germline control, variant allele frequency (VAF) estimation and comparing this to publicly available data sets or normal controls provide important clues to the possible germline origin of a variant. Based on historic observations on known germline variants and the VAF distribution patterns usually fall in the VAF range from 40% to 60% (heterozygous) or >80% (homozygous). Therefore, it is advisable to further evaluate a VAF >40% of a previously unannotated possible germline variant including correlation with available clinical history. It is also important to bear in mind approaches to help ensure the accuracy of VAF determination. For example, the gene function and nature of the mutation in tumour-suppressor genes often are associated with high VAF, even in the somatic setting, because of associated loss of the wild-type copy. Longitudinal assessment of repeated bone marrow aspirates that are routinely submitted form NGS can help determine whether the persistent of a high VAF clone despite treatment and disappearance of leukemic is suspicion is for germline origin (Figure 1) (DiNardo C et al, Cancer 2018)

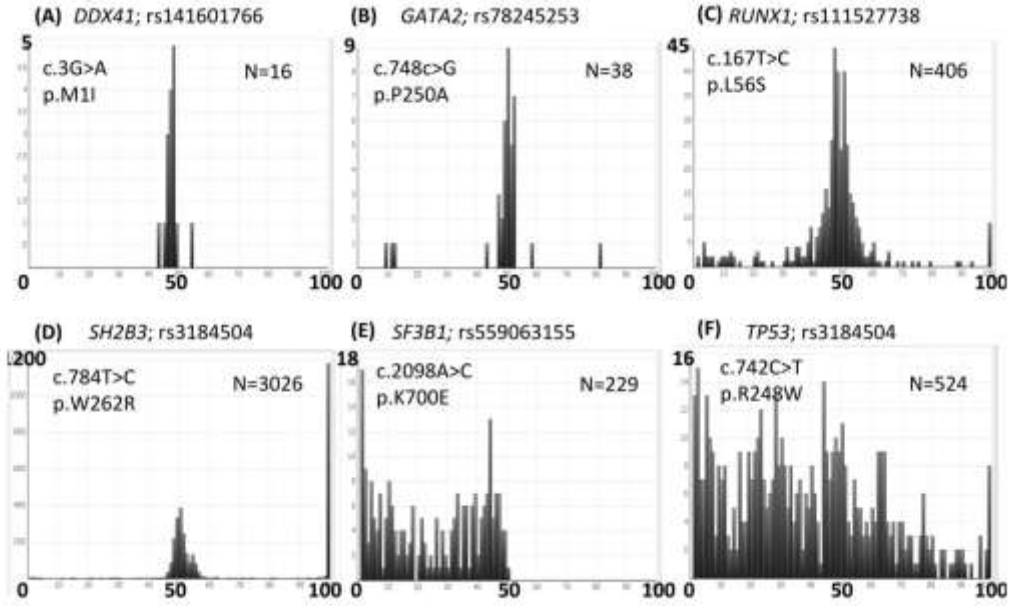

Figure 1. Illustrates the observed VAF distribution of various germline variants using NGS panel and comparison of a VAF in a suspected germline variant with known, well established somatic variants in the same sample.

Targeted sequencing

Customized development of targeted sequencing of genes of interest have been a growing trend and has helped to rapidly improve the detectability of FAML. Many of these panels are focused and include around 5-10 genes. In the study by Ana Rio-Machin et al, 2020; targeted sequencing of 10 known diseases genes (ACD, ANKRD26, CEBPA, DDX41, ETV6, GATA2, RUNX1, SRP72, TERC and TERT) was performed for index case of families with suspected FAML. Variants were categorised as being pathogenic if they have been previously described to be associated with familial AML/MDS or new variants such as single nucleotide variants with minor allele frequency in healthy population, variant allele frequency >30%, predicted to be pathogenic. In this study a cohort of 86 AMS/MDS families were investigated with 49 of these families harbouring germline variants in 16 previously defined loci of this gene panel (Rio-Machin et al Nature 2020).

Whole exome sequencing

NSG will only detect mutations from certain 'hot spots' pre-determined by the assays itself and probes e.g. FLT3 (exons 14 + 15 + 20), GNAS (exons 8 + 9), IDH1 (exon 4), IDH2 (exon 4). Therefore, this is not a genomic wide approach.

Whole exome sequencing (WES) provides a genome wide coverage compared to gene panel sequencing. Therefore, WES is often the preferred approach for exploratory studies of cancer genomes. Typically, WES

identifies about 18,000 to 20,000 nucleotide positions that are different from the reference genome, so called single nucleotide variants (SNVs), in usually a 54 Mbp covering the coding region of the human genome. Most, around 99% of these SNVs are known and are harmless single nucleotide polymorphisms (SNPs) and non-pathogenic germline SNPs which are specific to an ethnic group or the family. Thus, fewer than 0.1% of the SNVs are actually tumour specific somatic variants.

In order to identify these tumour specific somatic variants confidently, it is essential to know the germline SNPs which can be done by performing WES on DNA from a non-tumour tissue sample from the same proband (matched germ line control). In one study by Churpek et al, normal samples from 39 individuals from 7 families for germ line variants in the 264-gene WES panel. SNVs calling was performed by detailed bioinformatics and conservative filters using the software to produce a set of high-confidence germ-line variants excluding SNVs with VAFs of >5% in unaffected individuals and VAFs <30% in affected cases hence removing false-positive sequence artifacts and somatic variants representing tumor contamination of the normal sample. With this optimization they were able to detect novel candidates of germline alleles such as *HYDIN*, *MUC16*, *NMUR2*, *RNF213* (Churpek et al Blood 2015,)

Other techniques

It is important to note that NGS-based gene panels may not have been designed to identify large gene deletions, duplications, or structural variants. In addition, many pathologic germline variants occur outside of the transcribed exons, such as intronic variants in *GATA2* or 5′-promoter mutations in *ANKRD26*; thus, gene panels that fail to appropriately analyze these regions may provide false-negative results (Wlodarski MW, et al *Blood.* 2016).

Using *RUNX1* deletions as an example, array comparative genomic hybridization array (Array CGH) or SNP array and Multiplex Ligation-dependent Probe Amplification (MLPA) have been used to identify structional abnormalities that sequencing based technics fail to detect. In one study these techniques identified germline *RUNX1* deletions in the three families with clinical characteristics of familial platelet disorder with predisposition to myeloid malignancy (Rio-Machin et al Nature 2020).

Functional assays such as chromosome breakage test was the first test that should be used to diagnose Fanconi's anaemia is the chromosome breakage test, which is performed on a sample of the patient's blood in a clinical cytogenetics laboratory. The initial step involves culturing a sample of the patient's blood with a chemical substance known as a T-cell mitogen, which stimulates lymphocytes (a type of white blood cell) to divide. Next, the culture is treated with chemicals known as DNA cross-linking agents, such as mitomycin C (MMC) and/or diepoxybutane (DEB). Finally, the types and rates of breakages and rearrangements found in the chromosomes of cells are evaluated. Normal cells can correct most of the chromosomal damage caused by

the DNA cross-linking agents, whereas cells from patients with FA typically show multiple chromosomal breaks and rearrangements per cell (www.fanconi.org).

Ethical and psychological considerations of genetic testing

Genetic counseling is a critical component of the optimal care and management of patients who have a possible inherited cancer syndrome to help them better understand and adapt to the medical, psychological, and familial implications of genetic disorders. According to the study by Churpek *J et al*. 2013, all patients who undergo genetic testing should have a follow – up plan (ideally a face to face meeting with the geneticist) for disclosure of the test results once they become available. This is important because whether the results are positive or negative, a face to face counselling will help inform the patient about the meaning of their results, and the implications of the results on their health and any management plan based on the results or their family history. In case where the genetic test results are positive, the doctor/ geneticist should discuss the implications of these results with the patients and other family members who are at risk. Family members who are at risk would need to be given explanation regarding the non-haematological risks associated with some germline mutations and the potential risks and side effects associated with treatment. It is recommended that all mutation carriers consider undergoing bone marrow biopsy to assess for occult malignancy and twice a year a full blood count with differential testing (Churpek *J et al*. 2013).

Genetic disorders such as leukaemia do impact the physical, psychological and social well-being of not only patients but their families as well. Therefore, helping the patients and their families understand the purpose and the benefits of genetic testing can help guide them towards minimising distress and focusing on the benefits of the diagnosis (Alliance and Collaborative, 2020). The purpose of genetic testing is to identify carriers, predict leukaemia on set. However, it can cause psychological effects such as anxiety and depression. These effects often depend on the patients' perception of the risk, severity and controllability of the disease.

Patients diagnosed with genetic mutations can get to the stage where they consider themselves as "damaged" or broken. This can cause the patient to have a negative reaction, be frustrated and sometimes feel as if they are living with something that they cannot fight. In other situations, when a family member is diagnosed with mutations, other family members who are not affected can develop a sense of guilt because their relative is affected and they are not.

Summary

Doctors caring for patients with AML need to become familiar with the inherited predisposition syndromes underlying the development of the malignancy in certain families. Recognition of these syndromes is crucial to proper clinical management of patients with an inherited susceptibility and for genetic screening of

additional family members. Advanced molecular techniques such as NGS provides a significantly and continuously increasing our understanding of AML pathogenesis and treatment and has already led to changes to the prognostic stratification and disease classification by WHO.

For a broad use in daily clinical practice, standards for NGS based treatment decisions and monitoring have to be further defined in the future including at what timepoints during the course of treatment should NGS analysis be performed and which target genes should be included in a NGS panel and which sequencing coverage should be used. Germline mutations are being frequently added to this routine panel extending the possibility of identification. Along with this appropriate genetic counseling, family testing, and if appropriate, research studies should be carefully considered and the impact this has on the patient and family. Ethical considerations surrounding the rapid genetic methodological revolution and the identification of inherited cancer predisposition syndrome, often without any current clinically available preventative approaches, must be carefully discussed with the acknowledgment and respect of individual autonomy and informed consent.

Discussion

This dissertation summaries the clinical, biological and management challenges faced when considering a diagnosis of inherited AML in patients. A number of key conclusions can be drawn from this review.

Firstly, patients with inherited predisposition to AML are more common than previously recognized with an estimated incidence of 20%. Therefore, it is imperative to have additional detailed family history to identify if any family member has and a previous cancer, leukemia or hematological disorder. Pedigree mapping by genetics can facilitate the next steps in testing individuals within the family who are at risk. Additionally, as described in chapter 3 FAML can be associated with certain phenotypic and dysmphological features, therefore detailed examination is required.

Secondary, patients with inherited AML have very different clinical courses compared with patients with non-inherited AML. They are more likely to suffer toxicity and failures in standard therapy. In a nutshell, their leukemias can be more resistant to standard treatment and often aggressive treatment such as bone marrow transplantation is their only hope of cure. Therefore, identification upfront and in real time impacts the clinical care of these patients. This also impacts on family members as they may be habouring recessive genes that only come to light during a bone marrow transplant work up.

Thirdly, wide and high throughout genomic discovery platforms used in research to identify known genes and uncover new ones has to be balanced with validation studies and with functional assays, in some circumstances, as over or under interpretation of these can open up additional challenges for families. Additional, how these can be incorporated into routine affordable clinical testing settings is still in debate.

Finally, ethical considerations of the impact of identifications of suspected FAML genes on the patient and

family and role of genetic counselling involvement must be considered in order to fully support the physical, psychological and social well-being of patients and families.

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