Unlocking the potential of anti-CD33 therapy in adult and childhood Acute Myeloid Leukaemia (AML)

Acute Myeloid Leukaemia (AML) develops when there is a block in differentiation and uncontrolled proliferation of myeloid precursors, resulting in bone marrow failure. AML is a heterogeneous disease clinically, morphologically, and genetically, and biological differences between adult and childhood AML have been identified. AML comprises 15-20% of all children less than fifteen years diagnosed with acute leukaemia. Relapse occurs in up to 40% of children with AML and is the commonest cause of death.[1,2] Relapse arises from leukaemic stem cells (LSCs) that persist after conventional chemotherapy. The treatment of AML is challenging and new strategies to target LSCs are required. The cell surface marker CD33 has been identified as a therapeutic target, and novel anti-CD33 immunotherapies are promising new agents in the treatment of AML. This review will summarise recent developments emphasising the genetic differences in adult and childhood AML, while highlighting the rationale for CD33 as a target for therapy, in all age groups.

Differences in genetic profiles in adult compared to childhood AML

Adult and childhood AML differ significantly in the incidence and pattern of cytogenetic and molecular abnormalities, suggesting a different pathogenesis according to age.[3–5] Childhood AML, in comparison to adult AML, is characterised by a higher frequency of cytogenetic abnormalities creating fusion genes (e.g. *RUNX1-RUNX1T1*, *KMT2A*-rearrangements and *NUP98-NSD1*), specific chromosomal translocations e.g. t(1;22)(p13;q13)/*RBM15-MKL1*, t(7;12)(q36;p13)/*ETV6-MNX1* and t(11;12)(p15;p13)/*NUP98-KDM5A*) that occur almost exclusively in childhood and a lower frequency of genetic mutations (e.g. DNMT3A and IDH1/2). [6] (Table 1) Childhood AML also differs from adult AML in the incidence of AML arising from an underlying myeloproliferative or myelodysplastic condition. In children, the vast majority of patients present with de novo AML. In adults, there is a higher incidence of patients with an antecedent myeloid disorder e.g. myelodysplastic syndromes (MDS) and myeloproliferative neoplasm (MPN), and who meet the World Health Organisation (WHO) classification for AML with myelodysplasia-related changes.[7] Studies reviewing predominately adult populations, have found this group to account for 24-35% of all AML and it is associated with poorer outcome, increasing age, poor risk cytogenetic abnormalities and specific gene mutations.[8] For example, AML evolving from MDS has mutations involving splicing factors (SRSF2, SF3B1, UZAF1 and ZRSR2), epigenetic regulators (ASXL1, EZH2, BCOR) and members of the cohesion complex (STAG2).[7]

In adult AML, 40% of patients have a normal karyotype with increasing numbers of recognized driver mutations that allow patients to be categorised into prognostic groups, providing new potential therapeutic targets. Papemmanuil et al, have proposed a new genomic classification of adult AML. In addition to the existing WHO criteria, they suggest three additional categories identifying patients with mutations in chromatin remodelling and RNA splicing pathways (e.g. RUNX1, MLL, ASXL1, STAG2, DNMT3A, TET2), TP53 mutations and chromosomal aneuploidy, and AML with IDH2 mutations.[5] In addition, Ng et al, have identified a selection of prognostic biomarkers (LSC17 score) from genes differentially expressed in functional LSCs compared to the LSC negative AML fraction after correlation with clinical outcomes in adults.[9] The 17 genes include those associated with AML e.g. DNMT3B, SOCS2 and CD34, and additionally genes whose role in haematopoiesis is less clear. This score was shown to be able to identify high-risk patients with poor survival outcomes and response to treatments, including anti-CD33 antibody-drug conjugate (ADC) Gemtuzumab Ozogamicin (GO), across a spectrum of AML genotypes. They summarised that a high LSC17 score in patients reflected stemness properties that allow the LSCs to resist treatment, identifying patients as poor risk. These recent developments in genomic classification are helpful in progressing our knowledge, treatment plans and risk stratification for adult patients.

Childhood AML has been shown to have a different genetic landscape and the same classification systems may not be applicable to paediatric patients. Only 20% of paediatric patients have a normal karyotype and the number of somatic mutations is lower than in adult AML (5 per paediatric sample versus 10-13 per adult sample).[4] Shiba et al recently published whole exome sequencing and targeted sequencing of paediatric patients. In their cohort, they identified three major categories of recurrently mutated genes in addition to CEBPA (11%), WT1 (7.8%) and NPM1

(3.4%); these were the cohesion complex (RAD21, SMC3 and STAG2), epigenetic regulators (ASXL2, BCOR/BCORL1, EZH2), and signalling molecules (NRAS, KRAS, KIT, MPL, FLT3-ITD). ASXL1/2 mutations were seen in 8.8% of the cohort and 61% had co-existing t(8;21)(q22;q22). In contrast to adult patients, where this mutation is associated with poor outcomes, the additional ASXL1/2 mutation in children in this cohort did not result in a change of the favourable overall survival associated with t(8;21) alone.[4] No mutations where detected in the epigenetic modifiers, IDH1 or DNMT3A and mutations were rarely seen in IDH2, TET and BRAF; all of which are frequently mutated in the adult population. This illustrates some of the major differences between the genetic abnormalities in childhood and adult AML, with different frequencies of common mutations and unique mutations within each group.

Another potential difference in the pathogenesis of childhood and adult AML is the developmental properties of the cell of origin. There is evidence that the age and microenvironment of the cell of origin plays a significant role in the initiation and phenotype of a resultant leukaemia following genetic hits.[3,10,11] The intrinsic and extrinsic properties of haematopoietic stem cells (HSCs) change in the normal ageing process. For example, fetal liver HSCs have enhanced expression of self-renewal and cell cycling genes compared to adult HSCs. Adult normal HSCs are quiescent cells that show different gene expression profiles with increasing age. For example, there is upregulation of genes involved in myeloid lineage determination, DNA repair and cell death in HSCs from adults over 65 years compared to younger adult HSCs.[3] In the fetus, HSCs are first identified at the late stages of mid-gestation within the aorta-gonad-mesonephros (AGM) region and then move to the fetal liver and spleen before finally settling within the bone marrow niche just before birth.[12] The normal fetal HSCs therefore transition through a different microenvironment and are exposed to different extrinsic factors that may influence the response of the cell of origin to a driver mutation. The cell of origin or leukaemia initiating cell in AML does not always arise from the pluripotent HSCs, and it is important to consider the inherent properties and age related changes of normal haematopoiesis when considering leukaemogenesis.

Despite the differences discussed between adult and childhood AML, the main genetic driver often dictates the phenotype and prognosis of the disease, regardless of the age of the patient. For example, the cryptic NUP98-NSD1 translocation is found more frequently in children than adults. However, patients of all ages with this translocation present with higher white blood cell counts, have a specific gene expression signature and very frequently also harbour FLT3-ITD and WT1 mutations compared to NUP98-NSD1 negative cases.[13] The presence of NUP98-NSD1 was also an independent predictor for poor prognosis, with 4 year event free survival being less than 10% for both childhood and adult AML.[13]

The differences in the pathogenesis of adult and childhood AML is important clinically as to date, treatment of childhood AML is largely extrapolated from adult regimes. In the future, as we aim for a precision medicine approach, the differences in adult and childhood AML need to be considered to allow appropriate prognostic classifications and effective treatments to be allocated.

Table 1. Incidence of genetic abnormalities with age and associated CD33 expression and immunophenotype. [14–41]

The role of the LSC in AML

Over the past two decades, development of laboratory techniques has allowed the identification and characterisation of LSCs, primarily in adult AML. LSCs are a small population of cells that are capable of self-renewal as well as producing the 'bulk' AML blasts. It is thought that like normal haematopoiesis, there is an organisational hierarchy in AML, with LSCs at the top producing more differentiated leukaemic progeny.[42,43] LSCs have similar properties to normal HSCs, including quiescence, self-renewal capabilities and engraftment potential. LSCs are more complex however, with aberrant expression of cell surface markers, for example CD33, CLL-3, CD123 and CD45RA.[44–47] This may be explained by the theory that LSC are derived from more mature multi or unipotent progenitor cells that have acquired transforming mutations, providing the capacity for sustained self-renewal.[48] LSCs are also thought to be biologically different to the AML blasts that they produce. LSCs are quiescent, particularly in the G0 phase of the cell cycle and may home to the

bone marrow microenvironment, avoiding apoptosis and elimination with conventional therapies that target proliferating cells.[49]

It remains a cause of debate as to whether the causative mutations and subsequent creation of the LSC develop at the stage of multipotent HSCs or more mature myeloid progenitors.[50] The LSC population has traditionally been thought to be found in the CD34+/CD38- population.[51,52] This was based upon serial transplantation studies, performed using nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice, which showed that only the CD34+CD38- cells were capable of self-renewal.[52] However, several xenotransplantation studies using human adult AML samples, have found all CD34/CD38 positive and negative fractions can contain functional LSC that engraft, initiate and maintain the leukaemic process in immunodeficient mice.[9,42,44,49,50,53,54] It is important to note, that the ability of the leukaemic cells to engraft is highly dependent on the immunodeficient strain of the mice used. For example, Taussig et al, showed that CD34+CD38+ cells contained leukaemia-initiating cells using immunodeficient NOD/SCID/β_2-microglobulin null (NOD/SCID/β_2m $^{-/-}$), and NOD/SCID/IL2 receptor γ chain null (NOD/SCID/IL2rγ$^{-/-}$) mice treated with human immunoglobulin or anti-CD122 antibody to increase immunosuppression.[55] Therefore, the mice used in this study were more immunodeficient than the NOD/SCID mice used in earlier studies.

Goardon et al, provided evidence to show that the LSC in human adult AML can arise from a CD34+CD38- multipotent progenitor (lymphoid primed multipotential progenitors (LMPP)-like) present in the haematopoietic hierarchy below the level of the HSC and MPP, and a CD34+CD38+ unipotent progenitor population (granulocyte-macrophage progenitor (GMP)-like).[56] In addition, it has been shown that 25% of AMLs are CD34 negative (defined as less than 2% CD34+ cells).[48] Quek et al, report that CD34- LSCs most closely resemble granulocyte-macrophage precursors although they also express multiple normal HSC transcriptional regulators associated with self-renewal.[48] However, there are some AMLs that have both a CD34+ compartment (2-20% of total blast population) and CD34- compartment (up to 98%) that are not classed as a CD34- AML. Both compartments contain functional LSCs and illustrate that multiple clones can be present within individual AMLs.[48,56,57]

Papemmanuil et al, showed that 86% of adult patient AMLs had 2 or more driver mutations with patterns of co-mutation.[5] They confirmed the hypothesis that initial mutations develop in the founding clone e.g. DNMT3A, TET2, IDH1/2, and are followed by later transforming mutations e.g. NPM1, causing overt disease.[5] It has also been shown that in childhood AML specific translocations e.g. t(8;21), develop prenatally but do not necessarily result in overt leukaemia and a second event post-natally is most likely required for transformation to AML.[58] Initial mutations or translocations generate quiescent pre-leukaemic stem cells with the potential to form a founding clone but require further driver mutations to develop overt AML. It may be the case, that the secondary genetic or epigenetic mutations are acquired in the more mature progeny of the preleukaemic stem cells.[50] (Figure 1)

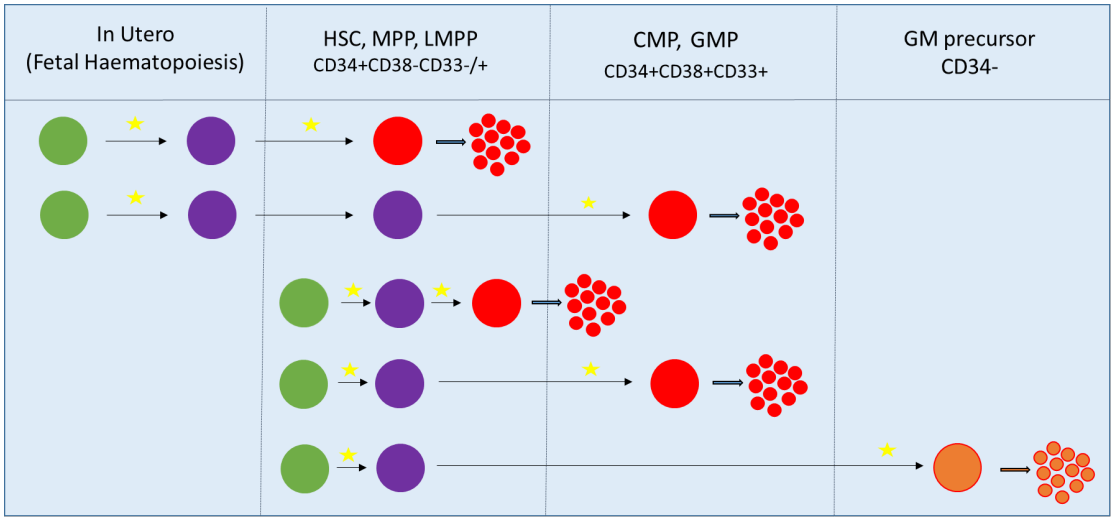

Figure 1: Summary of generalised models describing multi-step process in AML development from cells at different stages of maturity
The green circles represent normal cells within in the fetal liver or bone marrow in utero or in the bone marrow after birth at different stages of maturity: haematopoietic stem cell (HSC), multipotent progenitor (MPP), lymphoid primed multipotent progenitor (LMPP), committed myeloid progenitor (CMP), granulocyte-macrophage progenitor (GMP) or granulocyte-macrophage (GM) precursor. A star represents one or more insults that results in a change within the cell or microenvironment e.g. translocation or genetic or epigenetic mutation. An initiating mutation results in the development of a preleukaemic stem cell (purple) that gains self-renewal capacity. Further transforming mutations occurs leading to development of the LSC (CD34+ red, CD34- orange) that can produce the bulk AML blasts (CD34+ red CD34- orange).[46,48,50,56] There are exceptions to the proposed models in this figure. For example, MLL-fusion genes alone can be sufficient to cause infantile AML without a second genetic hit.[58]

CD33 expression in AML

CD33 is a member of the sialic acid binding immunoglobulin(Ig) like lectins (Siglecs), a subset of the immunoglobulin superfamily molecules.[42,59] CD33 is an attractive therapeutic target in AML due to the frequent expression of this transmembrane glycoprotein on adult and childhood AML blasts and importantly identified on adult LSCs. Extracellularly, CD33 has two Ig-like domains: V set Ig like domain and C2 set Ig-like domain.[59,60] Intracellularly, the cytoplasmic tail has one immunoreceptor tyrosine based inhibitory motif (ITIM) and a second ITIM-like tyrosine residue. (Figure 2). CD33 has endocytic properties when bound by bivalent antibodies that result in internalisation of the antigen/antibody complex. Therefore, binding of an antibody reduces the cell surface expression of CD33 but new CD33 sites are continuously expressed.[59,61] Alternative splicing of RNA can produce CD33 transcript variants that results in changes in the structure of CD33, for example, a CD33 splice variant missing exon 2 results in loss of the V-set domain. This is important clinically, as the altered CD33 may no longer serve as a target for anti-CD33 directed therapy due to loss of epitopes.[59,62] Single-nucleotide polymorphisms (SNP) have also been identified in paediatric patients and may be associated with CD33 expression levels and response to GO treatment.[63] Intracellular mutations in the ITIMs have also been shown to impair the endocytic properties of the receptor and subsequent internalisation of the antibody-CD33 complex.[60] The function of CD33 in normal and AML cells remains largely unknown. There is increasing evidence that it negatively regulates inflammatory and immune responses through inhibitory effects on tyrosine kinase-driven signalling pathways.[59]

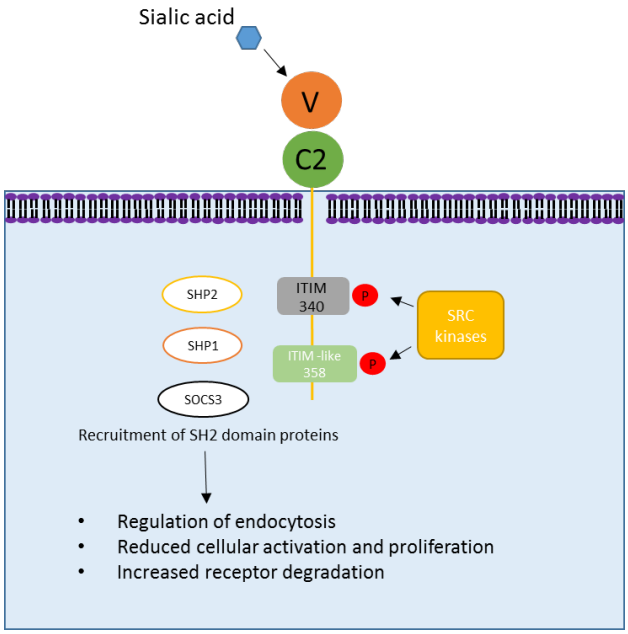

Figure 2: Structure and Signalling Pathway of CD33
67kd single pass transmembrane glycoprotein containing an amino-terminal V set Immunoglobulin- like domain that binds sialic acid and a C2 set Ig-like domain in its extracellular

portion. The cytoplasmic tail contains a tyrosine-based inhibitory signalling motif (ITIM) at position 340 and an ITIM-like domain at position 358. These domains are phosphorylated by SRC family kinases.[59] Once phosphorylated, they act as docking sites for SRC homology 2 domain containing proteins e.g. Src Homology 2 containing phosphatases 1 and 2 (SHP1 and SHP2). SH2 domain containing suppressor of cytokine signalling 3 (SOCS3) competes with SHP1 and SHP2 for binding to the ITIM.[50,59,64] SOCS3 has a SOCS box motif that allows it to form an E3 ubiquitin ligase complex. This results in the combined effects of regulation of CD33 endocytosis, reduced myeloid cell activation and proliferation and increased receptor degradation. SOCS3 however has been shown to reverse the CD33 induced inhibition of cell proliferation.[64]

CD33 is normally expressed on the myeloid lineage at the early stages of myeloid differentiation, being found on normal multipotent and unipotent myeloid progenitors, myeloid precursors and maturing granulocytes and monocytes.[59] The general consensus from the current literature, based on *in vitro* studies and multi-lineage engraftment of CD33-depleted autologous bone marrow transplants in patients, is that CD33 is not expressed on pluripotent HSCs.[42,43,45,59,65] However, there is a growing body of evidence to contradict this. Taussig et al, showed that $CD34^{+}CD38^{-}CD33^{+}$ healthy donor samples were capable of self-renewal by performing serial transplantation in immunodeficient mice, indicating that this fraction contains HSCs.[54] Krupta et al, more recently showed that CD33 was expressed on the $CD34^{+}CD38^{-}$ population of bone marrow from healthy donors but at a significantly lower intensity than that of LSCs.[19]

CD33 is expressed on blast cells in 85-90% of patients presenting with AML and also in the AML LSC fraction but with significant heterogeneity in the level of expression and intensity.[45,54,19] Studies in adults analysing CD33 expression at the level of the LSC ($CD34^{+}CD38^{-}$ fraction), showed that there were comparable levels of CD33 expression on bulk and LSCs in the majority, but not in all patients.[43,45] For example, if a patient highly expressed CD33 on blasts (>60%), the LSCs identified as $CD34^{+}CD38^{-}CD123^{+}$ cells co-expressed CD33. Conversely the LSC population (CD34+CD38-CD123+ cells) in a AML with CD33- blasts, were shown to be negative.[43] However, there is inter-patient variation and the level of expression and intensity of CD33 on $CD34^{+}CD38^{-}$ cells cannot be consistently predicted from CD33 expression on the 'bulk' AML blasts.

CD33 expression has been shown to vary between morphological, cytogenetic and molecular classifications of AML (table 1). FLT3-ITD, NPM1 and CEBPA mutated subgroups are consistently associated with high CD33 expression in both adult and childhood AML. Complex karyotypes and core binding factor (CBF) AML (t(8;21) and inv(16)) are associated with low CD33 expression in the bulk AML population.[66,45,19,18] Khan et al, interestingly showed in adult patients that the $CD34^{+}CD38^{-}$ fraction in core binding factor AML expressed CD33 while patients with intermediate and adverse risk disease, the $CD34^{+}CD38^{-}$ fraction was more heterogeneous, containing significant numbers of CD33 negative cells.[66] This suggests that CBF AML may have differences in expression patterns of CD33, and removal of CD33+ LSCs, may explain the favourable clinical response seen in adult patients receiving anti-CD33 therapy.

Gemtuzumab Ozogamicin in AML

Due to the high frequency of CD33 expression on AML blasts and importantly LSCs, the absence or lower CD33 expression on normal HSCs, and its endocytic properties, CD33 has been of interest as a therapeutic target. Gemtuzumab Ozogamicin (GO), an anti-CD33 ADC, was the first targeted treatment in AML to receive accelerated approval by the United States Food and Drug Administration (FDA) in 2000 for adults greater than 60 years with relapsed AML. This subsequently was voluntarily withdrawn in 2010 due to toxicity concerns. This was following a randomised control trial (RCT) performed by the South Western Oncology Group (SWOG) which assigned adult patients conventional first line chemotherapy with or without GO therapy. They found no clinical improvement but an increase in mortality with GO therapy. However, this RCT used different anthracycline doses in the two groups which may have negated the beneficial effects of GO and the mortality rate in the control group was lower than that normally expected. Meta-analysis data based predominantly on adult patients however, shows benefit from GO in selected patients, with core binding factor (CBF) AMLs showing the greatest benefit (overall survival benefit due to significant reduction in relapse rate).[67,68] The adult ALFA-0701 randomised control

trial, was an instrumental study altering the dosing schedule of GO, in an attempt to reduce toxicity, particularly the liver toxicity and risk of veno-occlusive disease (VOD), associated with GO. They gave GO in a fractionated dosing pattern, at a lower dose of 3mg/m^2 on days 1, 4 and 7 of induction chemotherapy and on day 1 of consolidation cycles.[69] Previously, GO was given at a higher single dose of 9mg/m^2. The rationale for this pattern of dosing was related to high levels of saturation of CD33 *in vitro* at a dose of 4mg/m^2 and the rapid re-expression of CD33 molecules on the cell surface.[69] The estimated 2 year event-free survival (40.8% GO vs 17.1% control), overall survival (53.2% GO vs 41.9% control) and relapse free survival (50.3% GO vs 22.7% control) were significantly improved in the GO group.[69] Severe adverse events remained higher in the group receiving GO, mainly due to persistent thrombocytopenia and two cases of VOD, but no difference in induction deaths were seen. Consistent with other RCTs, the benefit was most pronounced in patients with favourable cytogenetics i.e. CBF-AML. They also noted a pronounced benefit for patients with FLT3-ITD mutations compared to negative patients, therefore identifying a high-risk group of patients who respond to GO.

The Children's Oncology Group (COG) have also shown the benefit of GO in children with CD33 AML. The RCT AAML0531 compared paediatric patients being treated with conventional chemotherapy alone or in combination with GO, and found GO recipients had significantly improved event free survival at 3 years (53.1% GO vs 46.9% control) as well as relapse risk (32.8% GO vs 41.3% control) and disease-free survival (60.6% GO vs 54.7% control) in those receiving GO.[1,18] Pollard et al, analysed CD33 expression and clinical response to GO therapy in children. They found that patients with the lowest CD33 expression (quartile 1) who received GO had similar outcomes to those treated with conventional chemotherapy alone (EFS GO 53% vs 58% control, RR 36% GO vs 34% control).[18] Surprisingly 45% of this cohort had CBF AML, which as discussed have shown greatest response to GO.[18] Patients with CD33 expression within the higher quartiles (Q2-4) had higher complete remission rates for those who received GO therapy. High risk patients, with high CD33 expression treated with GO had a reduced relapse rate of 40+/-18%, compared to 73+/-22% in those who did not receive GO.[18] Therefore, in children, GO lacks clinical benefit in patients with lowest CD33 expression but reduces relapse rate in those with higher CD33 expression, regardless of cytogenetic risk group. [18] Khan et al, reviewed CD33 expression and response to GO in adult patients, and in contrast to the paediatric patients, CD33 expression was not independently prognostic for outcomes to GO.[66] There was a difference in the percentage of CBF-AML within the low CD33 expression group (45% of CBFs in Q1 in paediatric cohort vs 29% of CBFs in Q1 in adult cohort).[66] This shows that a higher proportion of children with CBF-AML in the COG cohort had low CD33 expression compared to the adult cohort. The findings again illustrate that childhood and adult AML have different genetic profiles, and that this can result in different responses to the same treatment. It emphasises the importance of investigating the specific paediatric responses to treatments, rather than assuming results can be transferable across the age groups.

GO has had a turbulent past but due to evidence of improved outcomes in selected patients and reassurance regarding toxicity, GO is gaining favour and is currently being investigated along with induction chemotherapy in a lower, fractionated dosing schedule in paediatric (Myechild01/NCT02724163) and adult (NCRI AML18/ISRCTN31682779 and AML19/ISRCTN78449203) AML in the United Kingdom. The above trials are currently recruiting patients and will close in November 2021, June 2019 and May 2020 respectively.

GO consists of a humanised murine IgG4 anti-CD33 antibody (clone hP67.6), linked via a hydrolysable bifunctional linker to the N-acetyl-γ-calicheamicin dimethyl hydrazide (CalichDMH).[59–61,70] GO binds to the surface CD33 and the complex is rapidly internalised. CD33 positivity is required *in-vitro* for GO-induced cytotoxicity, particularly at clinically achievable plasma levels.[54,60,71] The mean peak plasma level in an adult patient after the first dose of 9mg/m^2 GO is 3μg/ml.[60] CD33 is continuously re-expressed on the surface of the cells and allows further uptake of GO.[61] In the acidic conditions of the lysosome, the bifunctional linker is hydrolysed and the free CalichDMH is reduced to a diradical species, that binds to the minor groove of DNA, causing single and double-strand DNA breaks and a strong DNA damage response, with phosphorylation of the minor histone 2A variant (H2AX) and increased activation of DNA-dependent protein kinase (DNA-PK).[60,72] The IgG4 hP67.6 antibody alone is not associated with cell death but acts as the vehicle for drug delivery. Human cells exposed to GO *in vitro*, undergo cell cycle arrest at G2/M

phase and cells either undergo apoptosis or can escape cell death through DNA repair.[60,73] Apoptosis is thought to be independent of p53 and result from mitochondrial induced apoptosis. Proapoptotic proteins, BAX and BAK1 are thought to be important in sensitivity to GO, through formation of the mitochondrial permeability transition, release of cytochrome C and activation of caspase 3 and 9.[74,61,70] Members of the ATP-binding cassette (ABC) superfamily of transporter proteins, P-glycoprotein (Pgp/MDR1) and multi-drug resistance protein 1 (MRP-1) are thought to reduce sensitivity and DNA damage by efflux of CalichDMH out of the cell.[72] Yamauchi et al, created GO-resistant cell lines by incubating the human cell line HL-60 with increasing doses of GO over an 8 month time frame followed by limited dilution cell clone selection. They saw upregulation of DNA repair proteins at the gene and protein level (XRCC5, PARP1, RPA, GADD45A) compared to sensitive cell lines providing support for DNA repair pathways in resistance to GO.[72] They also provide evidence to suggest that CD33 expression levels, drug efflux transporters and upregulation of DNA repair pathways all contribute in part to the development of GO resistance (Figure 3).

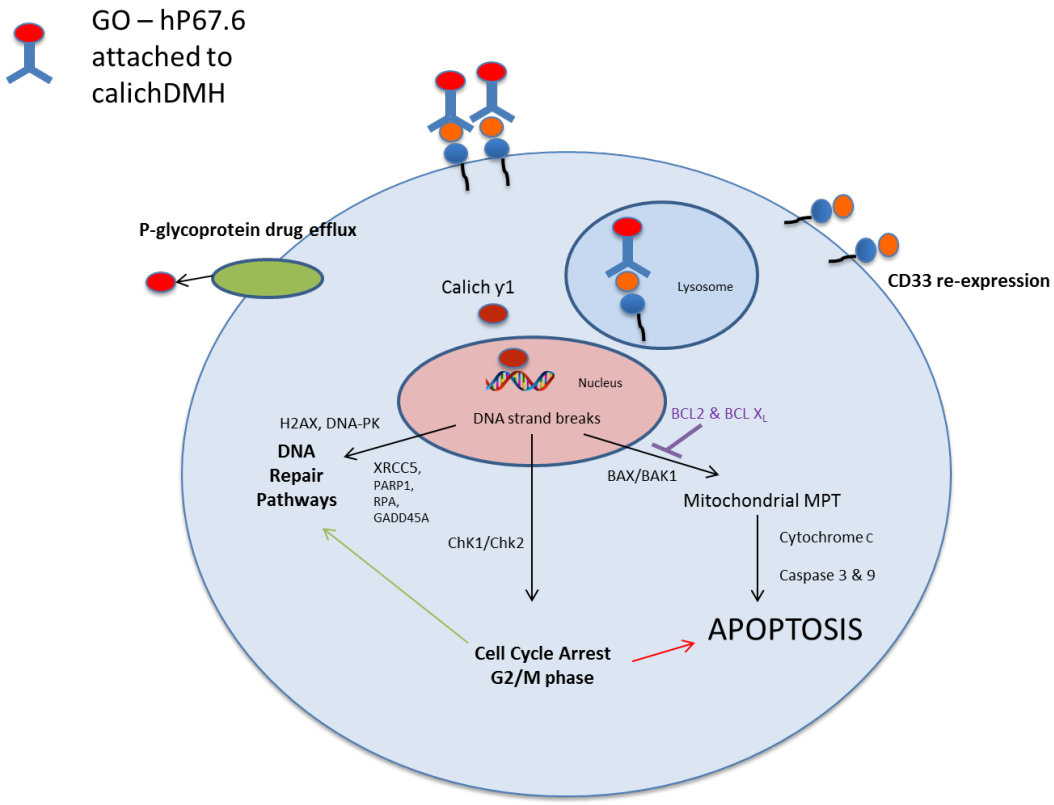

Figure 3: Summary of GO induced cytotoxicity and influencing factors.
GO attaches to CD33 on cell surface and the complex is internalised, where the bifunctional linker is hydrolysed within the lysosome. The calicheamicin γ1 derivative enters the nucleus, binds to DNA, and causes single and double strand DNA breaks. Free calicheamicin is pumped from the cell from drug transporters, e.g. P-glycoprotein. CD33 is continuously re-expressed. The DNA damage results in cell cycle arrest, DNA repair pathways and mitochondrial induced apoptosis. Cells entering cell cycle arrest can avoid apoptosis through upregulation of anti-apoptotic proteins ($Bcl2/BclX_L$) and DNA repair proteins (XRCC5, PARP1, RPA, GADD45A). MPT (mitochondrial permeability transition).

Future anti-CD33 therapies and treatment combinations

Monoclonal anti-CD33 antibodies have had less success clinically than their conjugated counterparts. The original anti-CD33 monoclonal antibody, lintuzumab showed good disease response in pre-clinical and early phase trials but did not improve survival outcomes in patients. A new Fc-engineered CD33 antibody, BI836858, is currently being tested in a phase I trial involving

adults with relapsed or refractory AML and those at high risk of relapse who are in complete remission (NCT01690624, estimated completion March 2019).

Vadastuximab talirine (SGN-CD33A) is a novel ADC against CD33. It consists of an anti-CD33 engineered cysteine antibody stably linked to a highly potent pyrrolobenzodiazepine (PBD) dimer. The ADC is internalised, transported to the lysosome where the linker is cleaved by proteolytic enzymes and the PBD dimer causes DNA damage and apoptosis through crosslinking DNA. It was shown pre-clinically to be effective against p53 mutated and multi-drug resistant AML.[75] Interim results from a Phase I trial have shown promising results with marrow blast clearance in 47% of adult patients with either relapsed AML or who declined conventional intensive chemotherapy, receiving single agent vadastuximab.[76] A further phase 1b study looking at vadastuximab in combination with standard induction chemotherapy showed a combined complete response (CR) and complete response with incomplete blood count recovery(CRi) rate of 74% with no significant increase in hepatotoxicity.[77] Recently, due to concerns regarding hepatotoxicity and VOD in the bone marrow transplant population, the FDA have placed a clinical hold or partial clinical hold on three vadastuximab phase I trials. The CASCADE trial is continuing to recruit.

A further ADC, IMGN779, is an anti-CD33 antibody conjugated to DGN462, an indolino-benodiazepine dimer with potent DNA alkylating properties. It has been shown to have in vitro and in vivo activity, particularly against FLT3-ITD mutated AML.[78,79] This ADC is being trialled in a phase I study in adult patients with relapsed/refractory CD33 positive AML (NCT02674763, estimated completion March 2019).

Bispecific T cell engaging (BiTE) antibodies are novel therapies that have shown success in other leukaemias e.g. blinatumomab in Acute Lymphoblastic Leukaemia. A CD33/CD3 BiTE, AMG 330, engages T cells with its CD3 binding site and brings the T cell alongside the AML blast via the CD33 binding site, resulting in T cell-induced cell death. Pre-clinical studies have shown that this drug is effective against CD33 expressing blasts, potentially avoiding the drug efflux drug resistance methods, and does not reduce CD33 expression through internalisation as seen with bivalent antibodies e.g. GO[80]. Phase I clinical trials in adults are now underway to investigate the safety and efficacy of this drug in patients with relapsed/refractory AML (NCT02520427, estimated study completion June 2018).

Chimeric antigen receptor targeting (CART) of CD33 is another exciting new approach to anti-CD33 therapy. This approach involves engineering T lymphocytes to express anti-CD33 chimeric antigen receptors, often with co-stimulatory inflammatory signals, resulting in T cell activation following contact with CD33 and resultant blast cell death. Pre-clinical studies in adults and paediatric AML human samples have shown excellent cytotoxic responses.[81,82] There are phase I trials underway in China (NCT02799680,NCT02944162: CAR-pNK including children >3 years). There is a major concern regarding the potential for prolonged, profound myelosuppression with this therapy and novel strategies to prevent this will be required.[82]

The combination of hypomethylating agents and anti-CD33 therapy, including GO, BI836858, vadastuximab and AMG-330, appears to be advantageous due to increased CD33 expression and increased sensitivity to DNA damage and apoptosis with hypomethylating agents in pre-clinical trials.[80,83–86] The combination of hypomethylating agents, azacitidine and decitibine, with vadastuximab is currently being assessed clinically in a randomised control trial recruiting older adult patients with newly diagnosed AML (CASCADE/NCT02785900, estimated completion date September 2021).

Summary

There is growing evidence that the pathogenesis of childhood and adult AML is different, and that they can respond differently to treatment. However, the main genetic driver, regardless of age, remains an important indicator of response to treatment and prognosis. CD33 is a promising target in both childhood and adult AML, as it is expressed on a variety of AML subtypes with different mutational landscapes, and clinical benefit has been shown with Gemtuzumab Ozogamicin (GO). This benefit is only seen in selected groups of patients and it is important to identify these patients to avoid unnecessary toxicity. The level of CD33 expression does appear to have a role in response,

particularly in children, but as clinical benefit is seen in cytogenetic groups with low CD33 levels, other factors must have a role in sensitivity. Drug efflux transporters, CD33 transmembrane structural variants, LSC response and upregulation of DNA repair pathways may all contribute to the effectiveness of GO. To enable further understanding and guide development of new generation CD33-directed drugs or drug combinations, further investigation into the response of the LSC, CD33 signalling pathways and resistance to CD33 therapies is required.

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References

1. Gamis AS, Alonzo TA, Meshinchi S, et al. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: Results from the randomized phase iII children's oncology group Trial AAML0531. *J Clin Oncol*. 2014. doi:10.1200/JCO.2014.55.3628.
2. Creutzig U, Heuvel-eibrink MM Van Den, Gibson B, et al. Diagnosis and management of acute myeloid leukaemia in children and adolescents: recommendations from an international expert panel. *Blood*. 2012;120(16):3187-3205-2012-03-362608. doi:10.1182/blood-2012-03-362608.
3. Chaudhury SS, Morison JK, Gibson BES, Keeshan K. Insights into cell ontogeny, age, and acute myeloid leukemia. *Exp Hematol*. 2015. doi:10.1016/j.exphem.2015.05.008.
4. Shiba N, Yoshida K, Shiraishi Y, et al. Whole-exome sequencing reveals the spectrum of gene mutations and the clonal evolution patterns in paediatric acute myeloid leukaemia. *Br J Haematol*. 2016;175(3):476-489. doi:10.1111/bjh.14247.
5. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med*. 2016;374(23):2209-2221. doi:10.1056/NEJMoa1516192.
6. de Rooij J, Zwaan C, van den Heuvel-Eibrink M. Pediatric AML: From Biology to Clinical Management. *J Clin Med*. 2015;4(1):127-149. doi:10.3390/jcm4010127.
7. Béné M, Nebe T, Bettelheim P, et al. REVIEW Immunophenotyping of acute leukemia and lymphoproliferative disorders: a consensus proposal of the European LeukemiaNet Work Package 10. *Leukemia*. 2011;25:567-574. doi:10.1038/leu.2010.312.
8. Devillier R, Gelsi-Boyer V, Brecqueville M, et al. Acute myeloid leukemia with myelodysplasia-related changes are characterized by a specific molecular pattern with high frequency of ASXL1 mutations. *Am J Hematol*. 2012;87(7):659-662. doi:10.1002/ajh.23211.
9. Ng SWK, Mitchell A, Kennedy JA, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. *Nature*. 2016;540(7633):433-437. doi:10.1038/nature20598.
10. Horton SJ, Jaques J, Woolthuis C, et al. MLL–AF9-mediated immortalization of human hematopoietic cells along different lineages changes during ontogeny. *Leukemia*. 2013;27(5):1116-1126. doi:10.1038/leu.2012.343.
11. Wei J, Wunderlich M, Fox C, et al. Microenvironment Determines Lineage Fate in a Human Model of MLL-AF9 Leukaemia. *Cancer Cell*. 2008;13(6):483-495. doi:10.1007/s11689-008-9001-8.A.
12. Babovic S, Eaves CJ. Hierarchical organization of fetal and adult hematopoietic stem cells. *Exp Cell Res*. 2014;329(2):185-191. doi:10.1016/j.yexcr.2014.08.005.
13. Hollink IHIM, Heuvel-eibrink MM Van Den, Arentsen-peters STCJM, et al. NUP98 / NSD1 characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct HOX gene expression pattern. *Blood*. 2011;118(13):3645-3656. doi:10.1182/blood-2011-04-346643.The.
14. Harrison CJ, Hills RK, Moorman A V., et al. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment Trials AML 10 and 12. *J Clin Oncol*. 2010;28(16):2674-2681. doi:10.1200/JCO.2009.24.8997.
15. Grimwade D, Hills RK, Moorman a V, et al. Refinement of cytogenetic classification in acute myeloid leukaemia: Determination of prognostic significance of rarer recurring chromosomal abnormalities amongst 5,876 younger adult patients treated in the UK

Medical Research Council trials. *Br J Haematol*. 2010;Conference(3):17. doi:10.1182/blood-2009-11-254441.Presented.
16. Balgobind B V, Raimondi SC, Harbott J, et al. Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. *Blood*. 2009;114(12):2489-2496. doi:10.1182/blood-2009-04-215152.
17. Grimwade D, Ivey A, Huntly BJP. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. *Blood*. 2015;127(1):29-42. doi:10.1182/blood-2015-07-604496.have.
18. Pollard JA, Loken M, Gerbing RB, et al. CD33 expression and its association with gemtuzumab ozogamicin response: Results from the randomized phase III children's oncology group trial AAML0531. *J Clin Oncol*. 2016;34(7):747-755. doi:10.1200/JCO.2015.62.6846.
19. Krupka C, Kufer P, Kischel R, et al. CD33 target validation and sustained depletion of AML blasts in long-term cultures by the bispecific T-cell-engaging antibody AMG 330. *Blood*. 2014;123(3):356-365. doi:10.1182/blood-2013-08-523548.
20. Khoury H, Dalal BI, Nevill TJ, et al. Acute Myelogenous Leukemia with t(8;21)—Identification of a Specific Immunophenotype. *Leuk Lymphoma*. 2003;44(10):1713-1718. doi:10.1080/1042819031000116698.
21. Hurwitz CA, Raimondi SC, Head D, et al. Distinctive immunophenotypic features of t(8;21)(q22;q22) acute myeloblastic leukemia in children. *Blood*. 1992;80(12):3182-3188. http://www.bloodjournal.org/content/80/12/3182.abstract.
22. Tiacci E, Pileri S, Orleth A, et al. PAX5 expression in acute leukemias: Higher B-lineage specificity than CD79a and selective association with t(8;21)-acute myelogenous leukemia. *Cancer Res*. 2004;64(20):7399-7404. doi:10.1158/0008-5472.CAN-04-1865.
23. Ossenkoppele GJ, van de Loosdrecht AA, Schuurhuis GJ. Review of the relevance of aberrant antigen expression by flow cytometry in myeloid neoplasms. *Br J Haematol*. 2011;153(4):421-436. doi:10.1111/j.1365-2141.2011.08595.x.
24. Orfao A, Chillón MC, Bortoluci AM, et al. The flow cytometric pattern of CD34, CD15 and CD13 expression in acute myeloblastic leukemia is highly characteristic of the presence of PML-RARα gene rearrangements. *Haematologica*. 1999;84(5):405-412.
25. Paietta E, Goloubeva O, Neuberg D, et al. A surrogate marker profile for PML/RARα expressing acute promyelocytic leukemia and the association of immunophenotypic markers with morphologic and molecular subtypes. *Cytometry*. 2004;59B(1):1-9. doi:10.1002/cyto.b.20001.
26. Falini B, Mecucci C, Tiacci E, et al. Cytoplasmic Nucleophosmin in Acute Myelogenous Leukemia with a Normal Karyotype. *N Engl J Med*. 2005;352(3):254-266. doi:10.1056/NEJMoa041974.
27. Lin LI, Chen CY, Lin DT, et al. Characterization of CEBPA Mutations in Acute Myeloid Leukemia: Most Patients with CEBPA Mutations Have Biallelic Mutations and Show a Distinct Immunophenotype of the Leukemic Cells | Clinical Cancer Research. *Clin Cancer Res*. 2005;11(4):1372-1379. doi:10.1158/1078-0432.CCR-04-1816.
28. Creutzig U, Harbott J, Sperling C, et al. Clinical significance of surface antigen expression in children with acute myeloid leukemia: results of study AML-BFM-87. *Blood*. 1995;86(8):3097-3108. http://www.ncbi.nlm.nih.gov/pubmed/7579404.
29. Muñoz L, Nomdedéu JF, Villamor N, et al. Acute myeloid leukemia with MLL rearrangements: clinicobiological features, prognostic impact and value of flow cytometry in the detection of residual leukemic cells. *Leuk Off J Leuk Soc Am Leuk Res Fund, UK*. 2003;17(1):76-82. doi:10.1038/sj.leu.2402708.
30. Lion T, Haas O a, Harbott J, et al. The translocation t(1;22)(p13;q13) is a nonrandom marker specifically associated with acute megakaryocytic leukemia in young children. *Blood*. 1992;79(12):3325-3330. http://www.ncbi.nlm.nih.gov/pubmed/1596573.
31. Peters JM, Ansari MQ. Multiparameter flow cytometry in the diagnosis and management of acute leukemia. *Arch Pathol Lab Med*. 2011;135(1):44-54. doi:10.1043/2010-0387-RAR.1.
32. Loghavi S, Zuo Z, Ravandi F, et al. Clinical features of De Novo acute myeloid leukemia with concurrent DNMT3A, FLT3 and NPM1 mutations. *J Hematol Oncol*. 2014;7(1):74. doi:10.1186/s13045-014-0074-4.
33. Li Y, Zhu B. Acute myeloid leukemia with DNMT3A mutations. *Leuk Lymphoma*. 2014;55(9):2002-2012. doi:10.3109/10428194.2013.869802.

34. Lu Q, Chen Y, Wang H, Li Z. DNMT3A mutations and clinical features in Chinese patients with acute myeloid leukemia. *Cancer Cell Int*. 2013;13(1):1. doi:10.1186/1475-2867-13-1.
35. Patel KP, Ravandi F, Ma D, et al. Acute myeloid leukemia with IDH1 or IDH2 mutation: Frequency and clinicopathologic features. *Am J Clin Pathol*. 2011;135(1):35-45. doi:10.1309/AJCPD7NR2RMNQDVF.
36. Medeiros BC, Kohrt HE, Arber DA, et al. Immunophenotypic features of acute myeloid leukemia with inv(3)(q21q26.2)/t(3;3)(q21;q26.2). *Leuk Res*. 2010;34(5):594-597. doi:10.1016/j.leukres.2009.08.029.
37. Chi Y, Lindgren V, Quigley S, Gaitonde S. Acute myelogenous leukemia with t(6;9)(p23;q34) and marrow basophilia: An overview. *Arch Pathol Lab Med*. 2008;132(11):1835-1837. doi:10.1043%2F1543-2165-132.11.1835.
38. Oyarzo M, Lin P, Glassman A, Bueso-Ramos C, Luthra R, Medeiros L. Acute myeloid leukaemia with t(6;9)(p23;q34) is associated with dysplasia and a high frequency of FLT3 gene mutations. *Am J Clin Pathol*. 2004;122(3):348-358.
39. Soupir CP, Vergilio JA, Dal Cin P, et al. Philadelphia chromosome-positive acute myeloid leukemia: A rare aggressive leukemia with clinicopathologic features distinct from chronic myeloid leukemia in myeloid blast crisis. *Am J Clin Pathol*. 2007;127(4):642-650. doi:10.1309/B4NVER1AJJ84CTUU.
40. Angelini DF, Ottone T, Guerrera G, et al. A Leukemia-Associated CD34/CD123/CD25/CD99+ Immunophenotype Identifies FLT3-Mutated Clones in Acute Myeloid Leukemia. *Clin Cancer Res*. 2015;21(17):3977-3985. doi:10.1158/1078-0432.CCR-14-3186.
41. Muñoz L, Aventin A, Villamor N, et al. Immunophenotypic findings in acute myeloid leukemia with FLT3 internal tandem duplication. *Haematologica*. 2003;88(6):637-645.
42. Pelosi E, Castelli G, Testa U. Targeting LSCs through membrane antigens selectively or preferentially expressed on these cells. *Blood Cells, Mol Dis*. 2015. doi:10.1016/j.bcmd.2015.07.015.
43. Hauswirth AW, Florian S, Printz D, et al. Expression of the target receptor CD33 in CD34 +/CD38 -/CD123 + AML stem cells. *Eur J Clin Invest*. 2007. doi:10.1111/j.1365-2362.2007.01746.x.
44. Jawad M, Seedhouse C, Mony U, Grundy M, Russell N, Pallis M. Analysis of factors that affect in vitro chemosensitivity of leukaemic stem and progenitor cells to gemtuzumab ozogamicin (Mylotarg) in acute myeloid leukaemia. *Leukemia*. 2010;24:74-80. doi:10.1038/leu.2009.199.
45. Ehninger A, Kramer M, Rö Llig C, et al. Distribution and levels of cell surface expression of CD33 and CD123 in acute myeloid leukemia. *Blood Cancer J*. 2014;439. doi:10.1038/bcj.2014.39.
46. Horton SJ, Huntly BJP. Recent advances in acute myeloid leukemia stem cell biology. *Haematologica*. 2012;97(7):966-974. doi:10.3324/haematol.2011.054734.
47. Kersten B, Valkering M, Wouters R, et al. CD45RA, a specific marker for leukaemia stem cell sub-populations in acute myeloid leukaemia. *Br J Haematol*. 2016;173:219=235. doi:10.1111/bjh.13941.
48. Quek L, Otto GW, Garnett C, et al. Genetically distinct leukemic stem cells in human CD34 − acute myeloid leukemia are arrested at a hemopoietic precursor-like stage. *J Exp Med*. 2016:jem.20151775. doi:10.1084/jem.20151775.
49. Pollyea DA, Gutman JA, Gore L, Smith CA, Jordan CT. Targeting acute myeloid leukemia stem cells: A review and principles for the development of clinical trials. *Haematologica*. 2014. doi:10.3324/haematol.2013.085209.
50. Walter RB, Appelbaum FR, Estey EH, Bernstein ID. Acute myeloid leukemia stem cells and CD33-targeted immunotherapy. *Blood*. 2012. doi:10.1182/blood-2011-11-325050.
51. Lapidot T, Sirard C, Vormoor J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*. 1994;367(6464):645-648. doi:10.1038/367645a0.
52. Bonnet, Dominique, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med*. 1997;3(7):730-737.
53. Kreso A, Dick JE. Evolution of the cancer stem cell model. *Cell Stem Cell*. 2014;14(3):275-291. doi:10.1016/j.stem.2014.02.006.
54. Taussig DC, Pearce DJ, Simpson C, et al. Hematopoietic stem cells express multiple myeloid markers: Implications for the origin and targeted therapy of acute myeloid

leukemia. *Blood*. 2005. doi:10.1182/blood-2005-03-1072.
55. Taussig DC, Miraki-moud F, Anjos-afonso F, et al. Anti-CD38 antibody mediated clearance of human repopulating cells masks the heterogeneity of leukemia initiating cells Anti-CD38 antibody mediated clearance of human repopulating cells masks the heterogeneity of leukemia initiating cells Corresponding aut. *Blood*. 2008;112(3):568-576. doi:10.1182/blood-2007-10-118331.
56. Goardon N, Marchi E, Atzberger A, et al. Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. *Cancer Cell*. 2011;19(1):138-152. doi:10.1016/j.ccr.2010.12.012.
57. Taussig DC, Vargaftig J, Miraki-Moud F, et al. Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34- fraction. *Blood*. 2010;115(10):1976-1984. doi:10.1182/blood-2009-02-206565.
58. Greaves MF, Wiemels J. Origins of chromosome translocations in childhood leukaemia. *Nat Rev Cancer*. 2003;3(9):639-649. doi:10.1038/nrc1164.
59. Laszlo GS, Estey EH, Walter RB. The past and future of CD33 as therapeutic target in acute myeloid leukemia. *Blood Rev*. 2014;28(4):143-153. doi:10.1016/j.blre.2014.04.001.
60. Linenberger ML. CD33-directed therapy with gemtuzumab ozogamicin in acute myeloid leukemia: progress in understanding cytotoxicity and potential mechanisms of drug resistance. *Leuk Off J Leuk Soc Am Leuk Res Fund, UK*. 2005;19(2):176-182. doi:10.1038/sj.leu.2403598.
61. Van Der Velden VHJ, Te Marvelde JG, Hoogeveen PG, et al. Targeting of the CD33-calicheamicin immunoconjugate Mylotarg (CMA-676) in acute myeloid leukemia: In vivo and in vitro saturation and internalization by leukemic and normal myeloid cells. *Blood*. 2001;97(10):3197-3204. doi:10.1182/blood.V97.10.3197.
62. Laszlo GS, Harrington KH, Gudgeon CJ, et al. Expression and functional characterization of CD33 transcript variants in human acute myeloid leukemia. *Oncotarget*. 2016;7(28). www.impactjournals.com/oncotarget.
63. Mortland L, Alonzo TA, Walter RB, et al. Clinical significance of CD33 nonsynonymous single-nucleotide polymorphisms in pediatric patients with acute myeloid leukemia treated with gemtuzumab-ozogamicin-containing chemotherapy. *Clin Cancer Res*. 2013;19(6):1620-1627. doi:10.1158/1078-0432.CCR-12-3115.
64. Orr SJ, Morgan NM, Elliott J, et al. CD33 responses are blocked by SOCS3 through accelerated proteasomal-mediated turnover. *Blood*. 2007;109(3):1061-1068. doi:10.1182/blood-2006-05-023556.
65. Robertson MJ, Soiffer RJ, Freedman a S, et al. Human bone marrow depleted of CD33-positive cells mediates delayed but durable reconstitution of hematopoiesis: clinical trial of MY9 monoclonal antibody-purged autografts for the treatment of acute myeloid leukemia. *Blood*. 1992;79(9):2229-2236. http://www.ncbi.nlm.nih.gov/pubmed/1571539.
66. Khan N, Hills R, Virgo P, et al. Expression of CD33 is a predictive factor for effect of gemtuzumab ozogamicin at different doses in adult acute myeloid leukaemia. *Nat Publ Gr*. 2016. doi:10.1038/leu.2016.309.
67. Loke J, Khan JN, Wilson JS, Craddock C, Wheatley K. Mylotarg has potent anti-leukaemic effect: a systematic review and meta-analysis of anti-CD33 antibody treatment in acute myeloid leukaemia. *Ann Hematol*. 2014;94(3):361-373. doi:10.1007/s00277-014-2218-6.
68. Hills RK, Castaigne S, Appelbaum FR, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: A meta-analysis of individual patient data from randomised controlled trials. *Lancet Oncol*. 2014;15(9):986-996. doi:10.1016/S1470-2045(14)70281-5.
69. Castaigne S, Pautas C, Terré C, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): A randomised, open-label, phase 3 study. *Lancet*. 2012. doi:10.1016/S0140-6736(12)60485-1.
70. Walter RB, Gooley TA, Velden VHJ Van Der, et al. Brief report CD33 expression and P-glycoprotein – mediated drug efflux inversely correlate and predict clinical outcome in patients with acute myeloid leukemia treated with gemtuzumab ozogamicin monotherapy. *Response*. 2007;109(10):4168-4170. doi:10.1182/blood-2006-09-047399.Presented.
71. Jawad M, Yu N, Seedhouse C, Tandon K, Russell NH, Pallis M. Targeting of CD34+CD38-cells using Gemtuzumab ozogamicin (Mylotarg) in combination with tipifarnib

(Zarnestra) in Acute Myeloid Leukaemia. *BMC Cancer*. 2012. doi:10.1186/1471-2407-12-431.
72. Yamauchi T, Matsuda Y, Tasaki T, et al. Induction of DNA strand breaks is critical to predict the cytotoxicity of gemtuzumab ozogamicin against leukemic cells. *Cancer Sci*. 2012;103(9):1722-1729. doi:10.1111/j.1349-7006.2012.02343.x.
73. Amico D, Barbui AM, Erba E, Rambaldi A, Introna M. Differential response of human acute myeloid leukemia cells to gemtuzumab ozogamicin in vitro : role of Chk1 and Chk2 phosphorylation and caspase 3. *Blood*. 2003;101(11):4589-4597. doi:10.1182/blood-2002-07-2311.Supported.
74. Haag P, Viktorsson K, Lindberg ML, Kanter L, Lewensohn R, Stenke L. Deficient activation of Bak and Bax confers resistance to gemtuzumab ozogamicin-induced apoptotic cell death in AML. *Exp Hematol*. 2009;37(6):755-766. doi:10.1016/j.exphem.2009.03.002.
75. Kung Sutherland MS, Walter RB, Jeffrey SC, et al. SGN-CD33A: a novel CD33-targeting antibody-drug conjugate using a pyrrolobenzodiazepine dimer is active in models of drug-resistant AML. *Blood*. 2013;122(8):1455-1463. doi:10.1182/blood-2013-03-491506.
76. Stein EM, Stein A, Walter RB, et al. Interim Analysis of a Phase 1 Trial of SGN-CD33A in Patients with CD33-Positive Acute Myeloid Leukemia (AML). *Blood*. 2014;124(21):623-623.
77. Erba HP, Levy MY, Vasu S, et al. A Phase 1b Study of Vadastuximab Talirine in Combination with 7+3 Induction Therapy for Patients with Newly Diagnosed Acute Myeloid Leukemia. *Blood*. 2016;128:211.
78. Whiteman KR, Noordhuis P, Walker R, et al. The Antibody-Drug Conjugate (ADC) IMGN779 Is Highly Active in Vitro and in Vivo Against Acute Myeloid Leukaemia (AML) with FLT3-ITD Mutations. *Blood*. 2014;124(21):2321.
79. Krystal WM, Walker R, Fishkin N, Audette C, Kovtun Y, Romanelli A. IMGN779, a CD33-Targeted Antibody-Drug Conjugate (ADC) with a Novel DNA-Alkylating Effector Molecule, Induces DNA Damage, Cell Cycle Arrest and Apoptosis in AML Cells. *Blood*. 2015;126:1366.
80. Laszlo GS, Gudgeon CJ, Harrington KH, et al. Cellular determinants for preclinical activity of a novel CD33/CD3 bispecific T-cell engager (BiTE) antibody, AMG 330, against human AML. *Blood*. 2014;123(4):554-561. doi:10.1182/blood-2013-09-527044.
81. O'Hear C, Heiber JF, Schubert I, Fey G, Geiger TL. Anti-CD33 chimeric antigen receptor targeting of acute myeloid leukemia. *Haematologica*. 2015;100(3):336. doi:10.3324/haematol.2014.112748.
82. Kenderian S, Ruella M, Shestova O, et al. CD33-specific chimeric antigen receptor T cells exhibit potent preclinical activity against human acute myeloid leukaemia. *Leukemia*. 2015;29:1637-1647. doi:10.1002/aur.1474.Replication.
83. Fathi A, Erba H, Lancet J, et al. SGN-CD33A Plus Hypomethylating Agents : A Novel , Well - Tolerated Regimen with High Remission Rate in Frontline Unfit AML. In: *American Society of Hematology Abstract*. ; 2015:1-2.
84. Sutherland MK, Yu C, Anderson M, et al. SGN-CD33A in Combination with Cytarabine or Hypomethylating Agents Demonstrates Enhanced Anti-Leukaemic Activity in Preclinical Models of AML. *Blood*. 2014;124(21):3739.
85. Kurimoto M, Matsuoka H, Hanaoka N, et al. Pretreatment of leukemic cells with low-dose decitabine markedly enhances the cytotoxicity of gemtuzumab ozogamicin. *Leukemia*. 2013;27(1):233-235. doi:10.1038/leu.2012.178.
86. Vasu S, He S, Cheney C, et al. Decitabine enhances anti-CD33 monoclonal antibody BI 836858-mediated natural killer ADCC against AML blasts. *Blood*. 2016;127(23):2879-2889. doi:10.1182/blood-2015-11-680546.

Table

Genetic abnormalities by age [14-17]	**Incidence in Children %**	**Incidence in Adults %**	**33 Expression Intensity** [18-19]	**Immunophenotype**	**Notes**
Risk group					
Good risk					
t(8;21)(q22;q22); RUNX1-RUNX1T1	14	7	CD33+ (weak)	CD34+, HLA-DR+, MPO+, CD13+, CD15+/CD65+, CD19+/-, PAX5+/-, CD79a+/-, CD56+/-	
inv(16)(p13.1q22)/t(16;16)(p13.1;q22)/CBFB-MYH11	7	5	CD33+(weak, increased heterogene	4+, CD4+, CD11b+, CD11c+, CD64+, CD36+, CD2+/- [23]	
t(15;17)(q22;q21)/PML-RARA	10	13	CD33+ (high)	CD34 low/absent , HLA-DR low/absent , CD117+/-, CD13+, CD15-, CD65-, CD64+, CD11a-, CD11b-, CD56+/- [23-]	
NPM1-mutated AML (normal karyotype) without FLT3-ITD	5	33	CD33+ (high)	CD34-, HLA-DR-, CD117+ (weak), CD123+,CD13+, MPO+, CD64+CD14+, CD11b+, CD68+	
CEBPA double mutation (normal karyotype)	11	4	CD33+ (high, heterogeneous)	CD34+, HLA-DR+, CD13+, CD65+, CD11b+, CD15+, CD14-, CD64-, CD7+ [27]	
t(1;11)(q21;q23)/MLL-MLLT11	Rare	Rare	NA	NA	Not good risk in all studies*
Intermediate risk					
t(9;11)(p22;q23)/MLL-MLLT3;t(11;19)(q23;p13.3)/MLL-MLLT1;Other MLL rearrangements	11	5	CD33+ (high, heterogeneous)	HLA-DR+, CD13-, CD14-, CD65+, CD4+ [28] Adult: CD34+/-, CD117+/-CD14+, CD4+, CD11b+, CD11c+, CD64+,	
t(1;22)(p13;q13)/ *RBM15-MKL1*	Rare	<0.5	CD33+	LA-DR+/-, CD34+/-, CD45+/-	Usually restricted to infants
DNMT3A mutations	0	15	CD33+	CD34+/-, CD11c+, CD14+, MPO+, CD13+, AML DNMT3A/FLT3/NPM1 : CD34+, CD13+, CD123+, CD3-, MPO+	associated with NPM1 mutations
IDH1/2 mutations	0	16	CD33+	CD117+, HLA-DR+/-, CD34+/-, CD38+, CD13+, MPO+/-, CD64+(FAB M5 only) [35]	
Cytogenetic abnormalities not classified as favorable or adverse	25	35	NA	NA	
Poor risk					
Monosomy 7	4	5	NA	NA	
Monosomy 5/deletion of chromosme 5 long arm (del(5q)	~2	~5	NA	NA	
inv(3)(q21q26.2) or t(3;3)(q21;q26.2)/RPN1-MECOM(EVI1-MDS1-EAP)	~2	~1	CD33+	CD13+, CD33+, CD117+, MPO+/-, HLA-DR+, CD34+, CD56+/-, CD7+/-36	
t(6;9)(p23;q34)/DEK-NUP214	~2	~1	CD33+	+/-, HLA-DR+/-, CD34+/-, TdT+/- [37-38]	
t(7;12)(q36;p13)/ETV6(TEL)-HLXB9(MNX1)	<1	0	NA	NA	restricted to infants
t(4;11)(q21;q23)/MLL-MLLT2(AF4);t(6;11)(q27;q23)/MLL-MLLT4(AF6);t(10;11)(p12;q23)/MLL-MLLT10(AF10)	5	2	NA	NA	
t(11;12)(p15;p13)/NUP98-KDM5A	~1	0	NA	NA	
t(5;11)(q35;p15.5)/NUP98-NSD1	~5	~2	NA	NA	mostly adolescents
inv(16)(p13.3q24.3)/CBFA2T3-GLIS2	<2	0	NA	NA	
t(9;22)(q34;q11.2)/BCR-ABL1	~1	~1	CD33+	4+, CD13+, CD19+/- [39]	
abnormal chromosome 17 short arm	3	~2	NA	NA	
abnormal chromosome 12 short arm	~4	~3	NA	NA	in adults, always seen in complex karyotype
WT1 mutation	8	9	NA	NA	
FLT3-ITD in absence of favourable cytogenetic/genetic features	10	25	CD33+ (high, heterogeneous)	CD34+/-, HLA-DR+, MPO+, CD36+, CD25+, CD123+, CD64+ CD99+, CD117 +/-, CD7+/-, CD11b+/-, CD4+/-	
Complex karyotype	13****	9***	CD33+ (weak, heterogeneous)	Variable	Not poor risk in children**

* Good risk in Balgobind et al, not confirmed in Harrison et al 2010

** Harrison et al 2010

***based on four or more abnormalities

NA- not available