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Harnessing the immune system in acute myeloid leukaemia

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Abstract

Acute myeloid leukaemia (AML) is an aggressive blood cancer caused by the proliferation of immature myeloid cells. The genetic abnormalities underlying AML affect signal transduction pathways, transcription factors and epigenetic modifiers. In solid tumours, it is emerging that the genetic landscape of the tumour has a direct effect on the anti-tumour immune responses and response to immunotherapeutic treatment. However, there remains little information as to whether genetic abnormalities affect anti-leukemic immune responses. This review discusses current knowledge of AML antigens and immune responses to AML with a particular focus on the role of T cells and natural killer cells. Understanding immune responses to AML has implications for the development and use of immunotherapies to treat AML patients with distinct genetic abnormalities.

Keywords Acute myeloid leukaemia; cancer immunosurveillance; immunoediting, neo-antigens; immunotherapy; immune checkpoints; immune suppression
1. Introduction

The recent success of immune checkpoint inhibitors such as ipilimumab, anti-CTLA-4 and nivolumab and pembrolizumab, anti-PD-1, in improving survival of metastatic melanoma patients highlights that the immune system can be successfully harnessed to target and eliminate cancer cells more broadly for clinical benefit [1-4]. An emerging paradigm for understanding cancer immunosurveillance and patient responses to immunotherapies is that genetic mutational quality directly correlates with tumour cell’s immunogenicity and is thus fundamental to driving patients’ clinical outcomes. Much of the evidence to support this paradigm has been generated from solid tumour patients such as melanoma, however less is known about the correlation of mutational quality and immune responses in haematological malignancies. This review will focus on acute myeloid leukaemia (AML) and discuss evidence for heterogeneous genetic abnormalities driving endogenous differential anti-leukemic immune responses. In particular, novel immunotherapeutic strategies will be discussed for treatment of AML patients.

The ability of the innate and adaptive immune cells to attack the tumour before it becomes clinically detectable is known as cancer immunosurveillance [5, 6]. However, cancers are able to avoid the immune response by a variety of mechanisms. Recently, “evasion of immune destruction” has been included as one of the emerging hallmarks of cancer [7]. Thus, the important role of the immune response in cancer control and progression warrants a brief summary of the current theories. The term "cancer immunoediting" was coined to describe the phases of the immune response to cancer [5, 8-10]. The elimination phase describes the initial recognition, targeting and killing of cancer by the innate and adaptive immune cells. The immune system and tumour cells may then enter an equilibrium phase where the immune system prevents the tumour from expanding. This phase is one of genetic instability in the tumour that eventually leads to the sculpting and escape of less immunogenic tumour cells from the immune system. The tumour cells facilitate escape either through employing mechanisms to suppress the immune response or by down-regulating (editing) immunogenic molecules. The next sections will discuss the evidence for the critical role of genetic mutations in cancer immunosurveillance.

2. Connecting oncogenesis and cancer immunosurveillance

The core feature of cancer cells that separates them from normal cells is the underlying genetic mutations that drive cancer progression [11]. Recently, a study identified 20 mutational signatures in 30 different types of cancer and found a varying prevalence of somatic mutations [12]. These mutational signatures may influence the ability of the immune system to recognise and attack the cancer. Proteins derived from mutated genes are known as neo-antigens. Interestingly, AML was found to have one of the lowest mutational burdens (somatic mutations per megabase of DNA) implicating AML cells as having low immunogenicity, one study found an average of only 13 mutations in genes of de novo AML patients [13]. However, as will be discussed the mutational quality rather than the mutational
burden may be more important. A number of studies have used sequencing data from solid tumours to investigate the relationship between genetic mutations and endogenous immune responses and have found correlations between certain genetic signatures and clinical outcomes [14-17].

It is necessary for neo-antigens peptides to bind to MHC Class I and be presented at the tumour cell surface in order to be immunogenic. In addition, the peptides have to be recognised as non-self by T cells through binding T cell receptors (TCRs). This is reflected in the finding that patients with mutations predicted to bind antigen presentation machinery, major histocompatibility complex (MHC) Class I have higher CD8+ tumour infiltrating lymphocytes and elevated expression of immune checkpoint markers, CTLA-4 and PD-1 indicating an elevated immune response and possible survival advantage [17]. Furthermore, investigation of the relationship between cytolytic activity and specific cancer mutations links higher cytolytic activity to neo-antigen expression and also suggests that cytolytic activity results in immunoediting of tumours cells with higher neo-antigen expression [16]. It follows that patients who exhibit high cytolytic activity and have immunogenic neo-antigens may benefit more from treatment with immunotherapies.

The importance of a patient's neo-antigen signature has been demonstrated by differential responses of melanoma patients to treatment with ipilimumab. A recent study showed that patients who responded to ipilimumab treatment had a specific neo-antigen signature derived from mutant proteins predicted to bind MHC Class I [18]. There has been less focus on neo-antigen burden in blood cancers compared to solid tumours, however, a study investigating the neo-antigen burden of 13 different cancer types by combining massive parallel sequencing and HLA-binding prediction algorithms showed that chronic lymphocytic leukaemia and AML had the lowest burden of neo-antigens generated from missense and frameshift mutations [19]. These data implicate leukaemias as potentially having low immunogenicity and thus difficult for antigen specific cytotoxic immune cells such as T cells to recognise.

Nevertheless, successful treatment of patients with haematological malignancies by allogeneic haematopoietic stem cell transplantation (allo-HSCT) shows that blood cancer cells can elicit and be eradicated by the immune system. Allo-HSCT was the most potent immunotherapeutic treatment available for decades. The anti-leukemic response in allo-HSCT transplant patients is mediated by a graft-versus-leukaemia (GVL) effect in which donor T cells and NK cells are able to eliminate cancer cells. The mechanisms of GVL effect have been extensively reviewed elsewhere [20-22]. While allo-HSCT is an important treatment strategy there are significant risks and morbidity associated with allo-HSCT including the development of graft-versus-host-disease, thus limiting this treatment to highly selected patient cohorts [23].

There is also evidence that the endogenous immune response can be effective in eradicating AML cells. This is shown by the positive correlation of lymphocyte recovery after
chemotherapy and low relapse rate [24]. In addition, rare cases of spontaneous remission (SR) in AML patients have been associated with activation of the immune system through pathogen infections [25]. Dissecting the mechanisms behind SR may be critical to understanding how the immune system can be targeted to eradicate AML cells.

3. Genetic heterogeneity in AML

As genetic mutational quality is important for activation of the anti-cancer immune response, at least in melanoma, knowledge of the genetics of AML will be important in dissecting the immune responses in leukaemia patients. AML arises from genetic abnormalities in hematopoietic stem cells (HSCs) giving rise to leukaemia stem cell (LSC) populations [26, 27]. Like HSCs, LSCs possess limitless self-renewal but have restricted differentiation capacity resulting in the proliferation of immature blast cells [28]. AML blasts have also been shown to disrupt normal haematopoiesis and inhibit the differentiation of normal progenitor cells [29]. Elimination of LSCs is critical to preventing relapse of AML patients [28]. The immune system may play a role in enabling the proliferation of AML blasts from LSCs. We refer the reader to a recent review on this topic [30]. Thus, understanding immune regulation of LSCs is important for the design of immunotherapies.

AML is a genetically heterogeneous disease with over 700 chromosome translocations identified so far, numerous gene mutations and aberrant expression of many genes [31, 32]. The current World Health Organisation (WHO) classification system incorporates genetics alongside morphology, cytochemical, immunophenotypic and clinical information to separate disease entities [33, 34]. The overall survival of AML patients and response to chemotherapy is primarily dependent on the patient's age and molecular cytogenetics or genetic abnormalities of the AML [35-39]. Most patients are over the age of 60 and in these patients, the long-term survival is less than 10%, identifying AML as one of the most lethal malignancies. In many patients, the molecular profile alone can be used to guide treatment strategy and predict long term outcome [39]. In addition, AMLs arising from treatment, that is, treatment-related AML (t-AML) and secondary AML (sAML) such as MDS-related and post-MPN AMLs generally have poor prognosis and distinct genetic profiles compared to de novo AML [40]. The scope of this review does not extend to a comprehensive review of AML genetics and current treatment strategies; however an excellent review article on this topic has been recently published [41].

The genetic mutations in AML tend to occur within a number of functional categorizations or groups including:

1. Activation of signal transduction pathways that result in increased proliferation of myeloid cells [42]. Common signal transduction pathways mutated in AML include FMS-like tyrosine kinase-3 (FLT3), Ras and c-KIT.

2. Aberrant activity of transcription factors that block differentiation of myeloid cells [43]. The aberrant activity can be caused by chromosomal translocations such as t(8;21)(q22;q22): RUNX1-RUNX1T1 (AML1-ETO) and t(15;17)(q22;q12): PML-RARα [44, 45].
3. Mutations in epigenetic modifiers. These include DNA methyltransferase 3A (DNMT3A), additional sex combs-like 1 (ASXL1), tet methylcytosine dioxygenase 2 (TET2), isocitrate dehydrogenase 1 (IDH1), IDH2, and enhancer of zeste homologue 2 (EZH2). These mutations cause widespread gene expression changes leading to enhanced self-renewal [46].

Genome-wide gene expression profiling of AML patients have revealed that AML oncogenic transformation results in widespread changes in gene expression [47-49]. Patients with distinct genetic abnormalities have distinct gene expression and epigenetic profiles [50-52]. AMLs with specific genetic abnormalities are also associated with certain morphological phenotypes and level of myeloid maturation [53]. This may be an important factor in immune recognition of different subtypes of AMLs because cell surface expression of proteins changes as myeloid cells mature.

The impact of oncogene-induced changes in gene expression on immune responses has recently been demonstrated in Hodgkin lymphoma. Alterations in the chromosome 9p24.1 causes over expression PD-1 ligands (PD-L1 and PD-L2) resulting in inhibition of T cell responses through interaction with the inhibitory T cell receptor PD-1 [54]. Treatment with nivolumab, anti-PD-1 therapy, showed objective responses in 87% of patients and linked response to therapy directly to the genetic alterations [55]. This important finding in Hodgkin lymphoma demonstrates the need for further research into the influence of genetic abnormalities on immune responses in AML.

Given the variation of genetic mutations in AML patients it will be informative to investigate if anti-leukemic immune responses are driven directly by recognition of neo-antigens, that is, mutated proteins; or if gene expression changes directly alter immune recognition proteins as in Hodgkin lymphoma. The next sections will discuss existing evidence for both scenarios.

4. Antigen presentation and activation of anti-leukemic immune response

4.1 AML antigen presentation

A critical step in the activation of the immune system is the recognition of neo-antigens presented at the surface of tumour cells. There are two main classes of antigen presentation machinery, MHC classes I and II. MHC Class I is encoded by a large group of polymorphic genes known as human leukocyte antigen (HLA) and is expressed on the majority of cells and presents self-antigen that is derived from cytosolic proteins to cytotoxic T cells. In contrast, MHC Class II expression is usually restricted to antigen presenting cells (APCs) and presents antigens derived from extracellular proteins to CD4+ T helper cells.

While AML cells are essentially precursors to APCs such as dentritic cells (DCs), they do not appear to have the same ability to generate an effective immune response [56, 57]. This may be due to defective formation of immune synapses and antigen presentation to T cells [58].
Interestingly, studies of MHC Class I expression on AML cells have shown that total loss of HLA molecules is infrequent [59-63]. This is in contrast to the loss or mutation of MHC Class I in solid tumours which can be a mechanism for immune escape from T cells [16, 64]. However, the sustained expression of MHC Class I may facilitate immune escape from NK cells as MHC Class I molecules are ligands for NK cell inhibitory receptors (killer cell immunoglobulin-like receptors (KIR)) (Figure 1).

The role of non-classical HLA molecules in AML is not well understood. Expression of non-classical HLA molecules has been linked with immune suppression in the tumour microenvironment [65]. HLA-G may be upregulated upon IFN-γ exposure to inhibit T and NK cell cytotoxic responses [66-68]. Increased soluble HLA-G was detected in the serum of acute leukaemia patients which may provide chronic inhibition (Figure 2C) [69].

In regards to MHC Class II, there is variable expression on AML cells [70, 71]. APL cells, characterised by the presence of the oncogene PML-RARα, consistently do not express MHC Class II [72]. The function of MHC Class II on other AML subtypes may be defective. A study showed that AML cells with MHC Class II bound to class II-associated invariant chain peptide (CLIP), a self peptide that is bound to MHC Class II before being switched for another antigen, had poor clinical outcome [73] (Figure 1). An examination of the immune responses to CLIP+ and CLIP- blasts revealed that CLIP+ blasts were less able to stimulate CD4+ T cells [74, 75].

In addition, MHC Class II expression on AML blasts may play a role in immune suppression rather than antigen presentation. Lymphocyte activation gene 3 (LAG-3), a negative stimulator of T cell activation [76], is known to bind MHC Class II and promote T cell tolerance [77, 78] (Figure 1). MHC Class II expression has been shown to protect melanoma cells from apoptosis through engagement with LAG-3 [79].

In the following sections, we discuss the roles of both AML-specific neo-antigens (ASNA) and AML associated antigens (AAA). ASNAs are proteins that arise directly from genetic alterations. AAAs are self-antigens that are aberrantly expressed by AML cells, have also been shown to be presented by MHC Class I [59] and elicit T cell responses in AML patients [80].

**4.2 AML-specific neo-antigens**

ASNAs, while specific to AML cells, may not be that effective in eliciting durable anti-leukemic immune responses. A number of ASNAs have been identified that are presented by MHC Class I, for example phosphopeptides [81]. Phosphopeptides may be important in AML and acute lymphocytic leukaemia (ALL) as more were identified compared to other types of leukaemia [81]. FLT3-ITD derived neo-epitopes were shown to be MHC Class-I restricted [82]. Subsequently, it has been demonstrated that AML blasts are capable of presenting FLT3-ITD neo-epitopes and inducing autologous T cell responses which may be important for allo-HSCT immune responses [83].
Some chromosomal translocations have been shown to induce CD4+ T cells responses. Specifically the PML-RARα fusion protein was shown to contain an antigenic site that is absent from the normal proteins and is recognised by CD4+ T cells [84, 85]. Similarly, BCR-ABL and DEK-CAN are also recognised by CD4+ T cells [86].

In cytogenetically normal AMLs, the mutated regions in NPM1 have been shown to generate both CD4+ and CD8+ T cell responses [87]. This suggests that the relatively favourable prognosis for patients with mutated NPM1 AML [88] may be due to the immunogenic neo-antigen properties [87].

### 4.3 AML associated antigens

Due to the low ASNA expression from AML cells, AAAs may represent promising immunotherapeutic targets. Currently identified AAAs have been previously reviewed [89].

Two recent studies have highlighted that the majority of AAAs are also expressed in normal healthy tissue [59, 90]. Nevertheless, AAAs are important targets for the immune system because many are involved in maintaining leukemogenesis [91]. For example, Wilms’ tumour protein 1 (WT1) is a zinc finger transcription factor that is over expressed in the majority of AML cases and importantly is increased in LSCs compared to normal HSCs, providing a target for the immune system to eliminate the quiescent population of malignant cells [92]. WT1 has been shown to block the differentiation of myeloid cells and may represent a promising candidate for antigen driven immunotherapies [93, 94].

Some AAAs may be associated with certain subtypes of AML [89]. A couple of studies directly link genetic abnormalities found in AML patients with the increased expression of AAAs. For example constitutive activation of FLT3-ITD and constitutively active tyrosine kinase BCR-ABL causes increased expression of AAAs and the stimulation of immune responses [95-97].

### 5. Immune responses in AML

#### 5.1 Defective anti-leukemic immune responses in AML patients and experimental models

The progression of AML in patients demonstrates that presentation of ASNAs and AAAs to the immune system is not sufficient to control AML expansion. AML cells employ many mechanisms to avoid immune destruction. The next sections will review defective cell-mediated cytotoxicity in AML patients and the immunosuppressive mechanisms employed to avoid the immune response. AML immune escape is caused by both intrinsic and extrinsic immunosuppressive mechanisms [10, 98]. Intrinsic immunosuppressive effects include upregulation of anti-apoptotic mechanisms, modulation of immunoregulatory checkpoints and loss of tumour antigen expression. Extrinsic mechanisms include accumulation of regulatory cells such as regulatory T cells (Tregs), tolerogenic lymphocyte phenotypes and secretion of immunosuppressive cytokines. The intrinsic tumour changes can directly
promote the extrinsic immunosuppressive mechanisms. The influence of mutational quality and quantity on immunosuppressive mechanisms is only starting to be elucidated. Manipulating these immunosuppressive mechanisms in AML may enable a more effective anti-leukemic immune response.

So far, the focus of this review has been on antigen-driven adaptive immune responses which involve mainly T cells and APCs. However, innate immune cells including NK cells and macrophages may also play critical roles in the anti-leukemic immune response. Defective cell-mediated cytotoxicity has been observed in AML patients and experimental models for both innate immune cells such as NK cells and macrophages and antigen-driven T cells [98].

Suppression of NK cell-mediated cytotoxicity demonstrated by the inability to lyse AML cells and produce cytotoxic cytokines is also an important immunosuppressive mechanism in AML patients [99-107]. Impaired NK cell-mediated cytotoxicity is demonstrated by. Impaired NK cell-mediated cytotoxicity has been linked with the expression of VEGFC on AML cells [108, 109]. In addition, circulating immature NK cells were shown to inhibit dendritic cell activation of T cells in AML patients [110].

In regards to the immunosuppression of T cells, there are substantial changes in the gene expression in T cells of AML patients compared to normal controls with aberrant activation profiles and the inability of T cells to form effective immune synapses with AML blasts [58] (Figure 1). Moreover, dysfunctional and exhausted T cells in AML are characterised by the co-expression of immunosuppressive proteins TIM-3 and PD-1 [111].

There is also an elevated frequency and higher proliferation of Tregs in newly diagnosed AML patients compared to healthy controls [112-114]. Tregs may facilitate AML blast expansion and relapse by accumulation in the bone marrow and suppression of T effector cells through secretion of immunosuppressive cytokines and increased ATP hydrolysis [112, 113, 115]. In addition, Tregs may also increase the production of adenosine, an immunosuppressant for T and NK cells [116]. One study showed that AML cells, themselves may, facilitate production of adenosine through expression of ectonucleotidases CD39 and CD73 [117].

Defective T cell-mediated cytotoxicity may be related to the secretion of immunosuppressive factors by AML cells. AML and myeloid derived suppressor cells (MDSCs) share many of the same molecular properties including indoleamine 2,3-dioxygenase 1 (IDO) expression [118-120], arginine metabolism [121], and secretion of reactive oxygen species (ROS) [122]. Importantly, elevated AML IDO expression was correlated with adverse prognosis [123-125]. Specifically, IDO, arginine metabolism and ROS are implicated in the inhibition of cytotoxic lymphocytes through the catabolism of amino acid tryptophan [118, 126-128], depleting arginine [121] and inducing apoptosis respectively [122, 129] (Figure 2D).
Figure 1: Defective antigen presentation and immune activation in AML. AML cells can express MHC Class I and present AML neo-antigens or AML-associated antigens to TCRs to activate T cell responses. However, AML cells can express B7 molecules, CD80 and CD86. These molecules bind inhibitory checkpoint molecule CTLA-4 on naive T cells to prevent co-stimulation. PD-L1 expression on AML cells may increase during relapse inhibiting cytotoxic T cell responses through binding PD-1 inhibitory checkpoint molecule. Ineffective synapse formation with AML blasts also inhibits T cell responses. MHC Class I on AML cells may also bind KIR inhibitory molecules on NK cells. Some subtypes of AML cells express MHC Class II and may activate CD4+ T cells responses. Binding of LAG-3 to MHC Class II inhibits TCR signalling. In addition, antigen presentation by MHC Class II may be prevented by the abnormal binding of CLIP molecule. TCR, T cell receptor; MHC, major histocompatibility complex; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; PD-1, programmed cell death protein 1; PD-L1/2, PD ligand; KIR, killer cell immunoglobulin-like receptor, LAG-3, lymphocyte activation gene 3.
5.2 Aberrant expression and activation of immune checkpoint molecules in AML

In addition to the secretion of immunosuppressive factors by cancer cells, the immune system is tightly controlled by immune checkpoint interactions, many of which are aberrantly expressed in cancer patients [130]. Next, we review the status of immune checkpoint activation in AML and explore connections with the mutational burden of leukaemia cells.

5.2.1 CTLA-4

CTLA-4 (Cytotoxic T-lymphocyte-associated protein 4) is an inhibitory receptor mainly expressed on T cells. It is thought that CTLA-4 inhibits T cell activation by out-competing co-stimulatory molecule CD28 binding to ligands CD80 (B7.1) and CD86 (B7.2) [130, 131]. Importantly, CTLA-4 binding occurs upon antigen engagement with the TCR. CTLA-4 was the first immune checkpoint to be targeted for cancer immunotherapy. Treatment of metastatic melanoma patients with ipilimumab, an anti-human CTLA-4 antibody, yielded improved survival in approximately 20% of patients [4].

AML cells have been shown to express the appropriate B7 molecules, CD80 and CD86, for engagement with CTLA-4 which may suppress activation of effector T cells [132-135] (Figure 1). AML differentiation status may affect susceptibility to CTLA-4 blockade as there are different expression levels of B7 molecules expressed on AML cells at different stages of maturation [132, 133]. In contrast, one study found that CTLA-4 is actually expressed by AML cells and that engagement with B7 molecules induces apoptosis of the leukaemia cells [136]. The benefit of CTLA-4 blockade in AML patients is currently under investigation and will be discussed in a later section.

5.2.2 PD-1/PD-L1

PD-1 (Programmed death receptor -1) is an important inhibitory immune checkpoint that suppresses the activity of T cells after antigen activation [137]. The ligands for PD-1 are PD-L1 and PD-L2. Evidence in other tumours suggests that PD-L1 is upregulated through exposure to inflammatory signals such as IFN-γ [130, 138]. In addition, PD-1 is highly expressed on Tregs and may aid in their proliferation [139]. PD-1 expression may inhibit the function of cytotoxic T cells in AML patients. Increased co-expression of PD-1 and TIM-3 on T cells was associated with an exhausted T cell phenotype in both a mouse model of disseminated AML [111] and in transplant patients before relapse [140].

In regards to PD-L1 expression, two murine AML models have shown that expression of PD-L1 on AML blasts facilitated immune escape and growth of the leukaemia [141, 142]. In AML patients, PD-L1 expression increases during relapse and during treatment indicating that immune suppression is an important mechanism in AML relapse and resistance to treatment [143-146] (Figure 1). There is inconclusive data for correlation of PD-L1 expression and AML subtype [143, 144, 147].

5.2.3 TIM-3/GAL-9
Another T cell inhibitory checkpoint is the TIM-3/GAL-9 interaction (T cell immunoglobulin mucin-3 and Galectin-9). As mentioned above TIM-3 expression is associated with exhausted T cells[111]. TIM-3 was more highly expressed on T cells in newly diagnosed AML patients compared to healthy controls and was associated with poor prognosis [148].

Interestingly, both TIM-3 and GAL-9 are expressed on AML cells [149-152]. TIM-3 is not expressed on normal HSCs but is expressed on LSCs in most AML subtypes apart from APL [153] and is associated with core binding factor translocations or mutations in CEBPA [151]. Stimulation of TIM-3 by GAL-9 upregulated pro-survival genes in AML cells and may promote expansion of myeloid-derived suppressor cells [152, 154] (Figure 2A). AML patients have significantly higher GAL-9 serum concentration compared to normal controls which may function as a positive feedback loop [152] (Figure 2C). Accordingly, TIM-3 may be a potential immunotherapeutic target as blockade of TIM-3 diminished leukemic burden in xenograft models [150].

5.2.4 NK cell-specific immune checkpoints

Immune cell ligands important for NK cell activation and inhibition are altered in AML patients. NK cell activity is controlled by the interaction of inhibitory receptors including KIRs and CD94/NKG2A with MHC Class I molecules on the surface of normal and tumour cells [155, 156]. Interestingly, leukaemia patients have an increased frequency of inhibitory KIR interactions compared to healthy controls [157, 158]. In addition, NK cells down-regulate natural cytotoxicity receptors (NCRs) in AML patients [159-161].

NKG2D is an activating receptor on NK cells. Ligands for this receptor can be expressed on tumour cells and upregulated by oncogenic stress, for example, the activation of the DNA damage response (DDR) proteins including ataxia telangiectasia mutated (ATM) [162, 163]. This may be important when the genetic mutation is initially created. NKG2D binds to a variety of ligands in both humans and mice [164]. In humans these ligands include MICA/B (MHC Class I polypeptide - related sequence A/B) and ULBPs (UL16-binding proteins). In mice the ligands are RAET1 (retinoic acid early inducible cDNA clone-1), MULT1 (murine ULBP-like transcript 1) and H60 family (histocompatibility antigen 60).

AML cells expressing NKG2D ligands, MICA and ULBP, are subject to cytotoxicity by NK cells [165, 166]. However, most studies show that AML cells express low levels of NKG2D ligands [165, 167-169]. Low level expression may be due to shedding as high levels of MIC have been detected in the serum of leukaemia patients [165] or methylation causing down-regulation of expression [170] to facilitate immune evasion of AML cells [171] (Figure 2C). Interestingly, treatment with cytarabine chemotherapy and development of drug resistance upregulated ULBP proteins making AML blasts susceptible to NKG2D-dependent NK cell-mediated lysis [166].

5.2.5 DNAM-1 and CD155/CD112 and CD96
DNAM-1 is an activating receptor for NK and T cells that is stimulated through interaction with CD155 (poliovirus receptor, PVR) and CD112 (Nectin-2). In AML, DNAM-1 can facilitate lysis of AML blasts [168, 169]. Interestingly, high levels of CD155 and CD112 have been reported on all AML subtypes in contrast to low levels of DNAM-1 on immune cells indicating a tolerogenic phenotype [168, 169, 172] (Figure 2B).

The ligand CD155 also interacts with another immunoregulatory molecule CD96. CD96 may also be an LSC restricted marker and a target for antibody therapy [173]. The role of CD96 in immune control is still under investigation; however, it was recently shown that CD96 binding CD155 dampens NK cell production of IFN-γ [174] implicating CD155 expression in immune escape of LSCs (Figure 2A).

5.2.6 Deregulation of tumour necrosis factor (TNF) superfamily and receptors

A number of members of the tumour necrosis factor receptor (TNFR) families and their ligands are expressed on AML cells and are associated with regulation of both T and NK cell responses.

TRAIL (TNF-related apoptosis-inducing ligand) is a member of the TNF superfamily found in various tissues including immune cells and can induce apoptosis of cancer cells [175]. TRAIL binds to a number of receptors, some of which are agonistic and others antagonistic or decoy receptors [175]. Studies have shown that AML blasts express both kinds of receptors, however co-expression of decoy receptors make blasts resistant to TRAIL-mediated apoptosis [176, 177] (Figure 2A).

Fas (fibroblast-associated ligand, CD95) and FasL (CD95L) are members of the TNFR and TNF families respectively. FasL is expressed by cytotoxic lymphocytes and binding of Fas by FasL can induce apoptosis of cancer cells [178]. AML patients have variable expression of Fas. Fas+ patients had increased relapse free survival. Conversely, newly diagnosed AML patients have greater FasL expression compared to normal controls [179]. This may be an immune escape mechanism as FasL can induce apoptosis of T cells [178].

CD137 (4-1BB) is a co-stimulatory molecule expressed on T and NK cells that binds CD137L (4-1BBL). Activation of CD137 increases T cell cytolytic activity [180]. CD137L is expressed on 35% of AML patients screened with the highest levels associated with monocytic AML subtypes [181]. Shedding of CD137L may constitute an immune escape mechanism as high levels of soluble CD137L (sCD137L) were found in the serum of AML patients and associated with worse prognosis [182, 183] (Figure 2C).

Glucocorticoid-induced TNFR-related protein ligand (GITRL) expression was found to correlate with monocytic AML subtypes and have low expression on undifferentiated AMLs [184]. In humans, GITRL was expressed on 50% of AML patients screened and GITR-GITRL interactions were found to impair NK cell-mediated cytotoxicity [184] (Figure 2A). The study of GITRL-GITR interactions is problematic as studies show that it has activating and inhibitory effects in mice and humans respectively [184-186].
5.2.7 Evidence for immunoregulatory molecules directly affected by genetic mutations

Specific mutations in Hodgkin lymphoma patients cause over expression of PD-1 ligands in the tumour thus inhibiting T cell responses. While expression of some of the immunoregulatory molecules mentioned in this section correlate with morphological phenotypes of AML cells there is no definitive evidence directly linking expression to genetic mutations. However, expression of CD200 and CD48 has been shown to be directly related to genetic mutations.

CD200 has been associated with immune suppression in the cancer microenvironment [187] including down-regulation of macrophage function [188], induction of Tregs [189] and suppressed NK cell activity in AML patients [190] (Figure 2A). CD200 may represent an important target for AML patients with chromosomal abnormalities including t(8;21) and inv(16). Microarray data showed that CD200 over expression was associated with these abnormalities and subsequent studies demonstrated that over-expression was caused by aberrant transcriptional activity of AML1-ETO [191, 192].

CD48 is the ligand for the activating NK receptor 2B4 (CD244). Both are downregulated in AML patients which may facilitate immune escape [172, 193] (Figure 2B). A study found that the oncoproteins, AML1-ETO and PML-RARα mediate down-regulation of CD48 through recruitment of histone deacetylase (HDAC) [194] although the clinical significance of this work is still to be determined.
Figure 2: Defective immune responses in AML patients. A. Expression inhibitory ligands. AML cells can upregulate inhibitory molecules FasL, TIM-3, TRAIL decoy receptors, GAL-9, GITRL, CD200 to bind corresponding ligands or receptors Fas, GAL-9, TRAIL, TIM-3, GITR and CD200R respectively and suppress the function of cytotoxic lymphocytes. In addition AML cells express CD47 which inhibits macrophage function by binding to SIRPα. Binding of TIM-3 on AML cells by GAL-9 may trigger pro-survival signals in AML cells and trigger MDSC expansion in myeloid cells. B. Downregulation of activating ligands and receptors. AML cells have been shown to express relatively high levels of ligands CD112 and CD155 but lymphocytes express low levels of the activating receptor DNAM-1 indicating a tolerogenic immune phenotype. There is low expression of activating ligand CD148 on AML cells and low expression of its receptors 2B4 on NK cells. C. Shedding ligands. A number of soluble ligands have been identified in AML patients. Soluble MIC (sMIC) molecules and soluble CD137L (sCD137L) may provide chronic stimulation to T and NK cells promoting a tolerogenic immune phenotype. Soluble HLA-G (sHLA-G), a non-classical MHC Class I molecule, may inhibit T and NK cells through binding KIRs on cytotoxic lymphocytes. Soluble GAL-9 (sGAL-9) may act simultaneously to promote AML cell survival through binding TIM-3 on AML cells and in addition inhibit T cell responses by binding TIM-3 expressed on T cells. D. Immunomodulatory enzymes and reactive oxygen species (ROS). AML cells have been shown to express IDO which catabolises the amino acid tryptophan inducing T cell suppression and apoptosis. Depletion of arginine by arginase II and the secretion of ROS have also been implicated in creating an immunosuppressive microenvironment and inducing apoptosis of cytotoxic lymphocytes. TIM-3, T cell membrane protein 3; TRAIL, TNF-related apoptosis-inducing ligand; HLA-G, human leukocyte antigen G; GAL-9, galectin 9; DR5, death receptor 5; MICA/B, major histocompatibility complex class I-related chain A/B; GITR, glucocorticoid-induced TNFR family related gene; GITRL, GITR ligand; IDO, indoleamine 2,3-dioxygenase 1; NKG2D, natural-killer group 2, member D; KIR, killer cell immunoglobulin-like receptor; SIRPα, signal regulatory protein α; MDSC, myeloid derived suppressor cells.
6. Harnessing the immune response to target AML

Novel immunotherapies have shown promise in the treatment of some haematological malignancies providing encouragement for their use in the treatment of AML [195, 196]. The evidence that patients can be cured through allo-HSCT (ie. positive responses in adoptive immunotherapy studies), and evidence of defective innate and adaptive immune responses in AML patients, all indicate that immunotherapies that boost immune responses against the leukemic cells could be effective treatment for patients with AML. Table 1 summarises selected current clinical trials underway to assess efficacy and benefit of immunotherapies in treating AML patients.

One of the challenges researchers face in the development of effective immunotherapies for the treatment of AML will be to overcome the many immunosuppressive mechanisms. Three broad categories of immunotherapies will be reviewed in subsequent sections: antigen-targeted immunotherapies for AML, immune checkpoint manipulation and cytokine therapies including adoptive transfer of NK cells.

6.1 Antigen-targeted immunotherapies for AML

Antigen-targeted immunotherapies use identified ASNAs and AAAs to generate specific immune responses to target AML cells. There are three main categories: leukaemia vaccines, the adoptive transfer of chimeric antigen-reactive T cells (CAR-T) and bispecific T-cell engager (BiTE) antibodies.

6.1.1 Leukaemia vaccines

Tumour vaccines are either peptide based vaccines or cell-based vaccines, both of which aim to direct the immune response to recognise tumour antigens. Clinical responses in AML patients to peptide vaccines using AAAs WT1, PR3 and RHAMM have been varied with some patients achieving complete remission [197-203]. Tumour vaccines may be most effective as consolidation therapy in combination with chemotherapy. For a full review of tumour vaccines targeting myeloid leukaemias please refer to Alatrash et al [204].

6.1.2 CAR-T cells

CARs are genetically engineered to re-direct the specificity of T cells to target specific antigens. The CAR-T cells are then adoptively transferred to leukaemia patients. CAR-T cells targeting CD19 have had promising clinical responses in the treatment of patients with B cell malignancies including B-cell lymphoma, chronic lymphocytic leukaemia (CLL) and B-acute lymphoid leukaemia (B-ALL) with treatment of B-ALL patients being the most successful [205-208]. In particular, one trial treating B-ALL patients reported a complete remission rate of 88% [209]. This review will not discuss the development and use of CAR-T cells in detail. However, two reviews have recently been published on this topic [210, 211].
The use of CAR-T cells in the treatment of AML patients may be more challenging as AML blasts share many antigens with early progenitor haematopoietic cells. There has been one clinical phase I trial assessing the efficacy of CAR-T cells engineered to recognise Lewis-Y (LeY) antigen to treat AML. One of the four patients achieved a transient cytogenetic remission but all patients eventually relapsed [212].

A number of CAR-T cells are in preclinical development including those targeting CD33, CD123 and WT-1. Preclinical studies of CD33 and CD123 CAR-T cells have shown effective targeting of leukemic blasts [213-215]. In one study, CD123 was found to have a safer profile against normal progenitor cells compared to CD33 [214]. However, another study showed that anti-CD123 CAR-T cells ablated normal human cells in xenograft mouse models [213].

WT-1 is another potential CAR-T cells target. Importantly, a small clinical trial reported that adoptive transfer of ex-vivo expanded WT-1 specific T cells isolated from bone marrow donors to post-transplant patients exhibited anti-leukemic activity [216]. Clinical trials are currently recruiting for anti-CD33 and anti-C123 CAR-T cells and for further investigation of WT-1 specific T cell approach (Table 1).

**6.1.3 Bispecific T-cell engager (BiTE) antibodies**

BiTE antibodies are formed by combining the binding domains from two separate antibodies. One domain binds the invariant chain of CD3 in order to recruit polyclonal T cells, and the other binds a tumour-specific antigen to force the formation of immunological synapses between the immune cell and leukemic cell. Blinatumomab, a BiTE antibody targeting CD19/CD3 in ALL patients has shown promising clinical benefits with clinical trials recording up to 80% minimal residual disease (MRD) response rate [217]. BiTE antibodies may also be effective in AML patients [218]. In particular, AMG330, a BiTE antibody targeting CD33/CD3 demonstrated preclinical efficacy against primary human AML cells [219]. Clinical trials in AML patients using BiTEs are in active planning.

**6.2 Immune checkpoint blockade**

Checkpoint blockade immunotherapies including anti-CTLA-4 and anti-PD-1 have revolutionised the treatment of metastatic melanoma patients. These treatments are now under investigation for other solid tumours and haematological malignancies. In regards to acute leukaemias the case for immune checkpoint blockade may be more complicated [196]. However, as previously mentioned, impressive results have been achieved through PD-1 blockade in the haematological malignancy Hodgkin’s Lymphoma with patients having an overall response rate of 87% [55]. The numerous immunosuppressive mechanisms as work in AML patients suggest that the immune system, if re-activated, may be able to target and kill leukaemia cells. The following sections will discuss the potential use of immune checkpoint blockade in the treatment of AML.
6.2.1 Anti-CTLA-4

Benefits of CTLA-4 blockade are currently under investigation in relapsed AML patients. Recent studies have shown that the primary mechanism of action of anti-CTLA-4 in solid tumour patients may actually be the depletion of intra-tumoral Tregs rather than direct action on effector cytotoxic T cells [220]. Depletion of Tregs in AML patients may be beneficial due to the increased Treg population in AML patients compared to normal controls [112-114]. Due to the immunosuppressive nature of AML, CTLA-4 blockade may be best used as a consolidation therapy post-chemotherapy treatment. There is currently a phase I clinical trial evaluating ipilimumab for AML patients with minimal residual disease (NCT01757639).

Preclinical mouse studies indicate that CTLA-4 blockade may also be beneficial to AML patients in the allo-HSCT setting and may assist the GVL effect [221]. Clinical trial, NCT01822509, is evaluating ipilimumab in patients with relapsed AML after allo-HSCT with progressive AML.

6.2.2 Anti-PD-1

Increased PD-1 expression on exhausted T cells and increased PD-L1 expression on AML cells suggests that PD-1 blockade may help to re-activate the anti-leukemic immune response [222]. Indeed, preclinical studies show that PD-1 blockade increased survival in a murine model of AML [142]. A Phase I clinical trial evaluating CT-011, a humanised antibody interacting with PD-1 for treatment of patients with late stage haematological malignancies showed clinical benefit for 33% of enrolled patients with one complete remission [223]. CT-011 is being further investigated in conjunction with a DC/AML vaccine following chemotherapy induced remission (NCT01096602).

6.2.3 Additional checkpoint blockade therapies

Anti-CD47

The emerging paradigm that neo-antigen quality is the driving factor behind clinical responses to immunotherapies implies that antigen-specific cytotoxic lymphocytes (CTLs) are the main effector cells driving anti-cancer immune responses, however, these concepts have been developed in neo-antigen rich tumours such as melanoma. In the case of cancers with low neo-antigen burden such as AML, the innate immune system including macrophages and NK cells may be important in driving successful anti-cancer immune responses [224, 225].

CD47 is the ligand for signal regulatory protein alpha (SIRPα), an inhibitory receptor expressed on phagocytic cells that prevents phagocytosis [226]. In AML patients, CD47 is more highly expressed on LSCs compared to normal HSCs and is correlated with adverse survival [225] (Figure 2A). High CD47 expression has been associated with NPM1 mutations [227] and FLT3-ITD [225] whereas patients with the favourable cytogenetic abnormality t(8;21)(q22;q22) had significantly lower CD47 expression [225]. Preclinical testing in AML
mouse models showed that a monoclonal antibody against CD47 facilitated phagocytosis of AML cells [225]. Phagocytosis of the cancer may then enable efficient priming of anti-tumour T cells [228]. The anti-CD47 humanized antibody, Hu5F9-G4 will be evaluated in clinical trials for solid tumour and for AML in the future [229] (NCT02216409).

**Anti-KIR therapies**

NK cell-mediated killing of AML cells may be enhanced by blocking KIR on NK cells. In allo-HSCT patients, KIR incompatibility, that is, inability of donor NK KIRs to recognise recipient HLA molecules, enhances NK-mediated anti-tumour efficacy and reduces relapse in AML patients [230]. This mechanism may be able to be harnessed by monoclonal antibody blockade of KIRs. Preclinical characterisation of an anti-KIR antibody, 1-7F9, increased killing of HLA-matched AML cells [231]. Another anti-KIR monoclonal antibody, IPH2101, was investigated in a phase I clinical trial for patients in first complete remission [232, 233]. Further analysis is needed to determine the benefits of KIR blockade in treatment of AML.

**6.3 Inhibition of immunosuppressive factors**

Inhibition of immunosuppressive factors such as IDO and ROS may enable a more efficient anti-leukemic immune response. In preclinical studies, IDO inhibitor, 1-methyl tryptophan (1MT), enhanced lymphocyte proliferation but did not affect AML blast proliferation. However, 1MT enhanced adriamycin inhibition of AML blast proliferation. This indicates that IDO inhibition may be most beneficial to clear MRD [234]. In addition, a small molecule inhibitor of IDO1, INCBO24360, resulted in increased lymphocyte numbers, reduced DC apoptosis and reduced conversion of naïve T cells to Tregs [235].

In regards to ROS, the inhibition of ROS through treatment of AML patients with histamine dichloride improved survival of AML patients in complete remission in a phase III clinical trial [236-238]. However, this approach has not been widely adopted by clinicians. Interestingly, post analysis of the clinical trial showed that patients with monocytic leukaemia had improved survival but not patients with myeloblastic AML [122]. This highlights the need to understand the mechanistic differences underlying immunosuppression in distinct AML subtypes.

**6.4 Cytokine therapies and adoptive transfer of NK cells**

Cytokine therapies are being investigated that enhance NK cell-mediated killing of cancer cells including AML [239, 240]. IL-2 may activate NK cells to enhance killing of tumour cells. A number of clinical trials have been conducted for patients in first complete remission; however meta-analysis of the trials concluded that IL-2 alone was not an effective maintenance therapy [241]. Preclinical studies indicate that it may be necessary to combine IL-2 with depletion of Tregs to achieve effective killing of AML cells [242]. Nevertheless, a further phase 4 clinical trial is being conducted combining IL-2 and histamine dichloride to inhibit ROS production (NCT01347996).
IL-15 is a critical cytokine for the development and homeostasis of NK cells and cytotoxic T cells. Preclinical studies of IL-15 stimulation of NK cells showed upregulation of activating receptors and enhanced cytotoxicity against autologous AML blasts [161]. A number of clinical trials are underway to investigate IL-15 as a potential therapeutic. A phase I clinical trial (NCT01385423) aims to transplant hapoidentical donor NK cells in combination with intravenous IL-15 in relapsed or refractory AML patients.

Other strategies include, activating NK cells prior to the infusion through exposure to the leukaemia cells (NCT01520558) [243]. A recent development in the NK cell immunotherapy field is the production of cytokine-induced memory-like NK cells (CIML) by exposure to IL-12, IL-15 and IL-18 before transfusion into patients [244]. This strategy is being investigated in relapsed AML patients in combination with chemotherapy conditioning and IL-2 infusion (NCT01898793).

Interferon-alpha (IFN-α) has also been shown to upregulate NK cell-mediated killing [245-248]. Administration of IFN-α has provided some clinical benefits to AML patients [249, 250]. Current clinical trials are investigating IFN-alpha’s potential to enhance the GVL effect after allo-HSCT (NCT02027064 and NCT02328755).

7. Challenges for immunotherapeutic treatment of AML

The relatively recent success of immune checkpoint blockade, such as anti-CTLA-4 and anti-PD-1, in solid tumours with high neo-antigen burden such as melanoma highlights the importance of cancer immunogenicity for the successful activation of the immune system and eradication of tumour cells. Thus, one of the main challenges facing the successful use of immunotherapies to treat AML patients will be the low neo-antigen burden and thus low immunogenicity of AML cells.

In solid tumours, the presence of tumour-infiltrating lymphocytes (TILs), especially CD8 T cells, is an indicator of good prognosis [251]. Interestingly despite low neo-antigen burden good lymphocyte recovery after chemotherapy is also an indicator of good prognosis for AML patients. Thus, immunotherapies boosting T cell response may still be effective in treatment of AML.

Part of the solution to AML’s low neo-antigen burden may lie in understanding how specific genetic abnormalities affect the immunogenicity and interaction with the immune system. As mentioned in this review, certain AML subtypes may be associated with certain immunosuppressive mechanisms such as upregulation of inhibitory molecules on the cell surface and secretion of immunosuppressive factors. For example, CD47 upregulation may be connected with FLT3-ITD mutation [225]. In this way, anti-CD47 therapy would be targeted to AML patients with FLT3-ITD mutation.

Designing antigen specific immunotherapies such as cancer vaccines and CAR-T cells may be another solution to overcome low neo-antigen burden. While this strategy has worked well for lymphocytic leukaemias, in particular ALL, unique antigens to target on AML cells may
be harder to find. As discussed earlier, many antigens are shared between AML cells and early haematopoietic progenitor cells. Thus, on target off tumour toxicity may result in destruction of the haematopoietic system.

Due to low neo-antigen burden, immunotherapies that boost the innate immune response may be more effective in eradicating AML cells than antigen-dependent mechanisms. In particular, treatments discussed in section 6 that boost NK cell and macrophage anti-leukemic responses are being optimised for the clinic. Of particular interest is the development of anti-KIR therapies inspired by potent GVL effects driven by hapoidential donor NK cells to clear residual AML cells.

Allo-HSCT still presents a critical part of the treatment strategy for AML patients. Optimisation of the GVL effect would present the most potent immunotherapeutic to eradicate AML cells. To enhance the GVL effect, allo-HSCT may be combined with a variety of immunotherapies including immune checkpoint blockade and cytokine therapies to focus a more potent GVL effect.

Another major challenge for immunotherapeutic treatment of AML is overcoming the potent immunosuppressive mechanisms at work in AML patients. One way to overcome the multiple immunosuppressive mechanisms is to combine immunotherapies. The benefits for combining immune checkpoint inhibitors anti-CTLA-4 and anti-PD-1 can be clearly seen in melanoma patients where combined checkpoint blockade increases advanced melanoma patient survival compared to treatment with only one agent [4, 252, 253]. In a preclinical AML murine model, combined immune checkpoint blockade was used alongside adoptive T cell immunotherapy. Importantly, it was found that blockade of PD-1, CTLA-4 and LAG-3 immune checkpoints were required for the adoptive T cells to exert anti-leukaemic effects [254]. In addition, pre-clinical studies have also suggested that PD-1 blockade be used in combination with BiTEs to enhance lysis of AML cells [255].

For the immune response to be re-activated against AML cells, it will not only be necessary to block checkpoints on immune cells, it will also be necessary to disable the immunosuppressive mechanisms exerted by the AML cells themselves, for example, increased ROS and IDO secretion. In addition, the immune cells may need a helping hand to become activated. This could be achieved through the transfusion of cytokines such as IL-2 and IL-15.

Due to low neo-antigen burden and potent immunosuppression, immunotherapies are unlikely to be highly effective in patients with full-blown AML. It may be necessary to reduce the bulk of the AML cells with chemotherapy before treatment with immunotherapies is effective. This strategy may also assist in the recovery of patients’ normal immune systems through the blocking of immunosuppressive mechanisms.

It remains to be seen whether the immunotherapies discussed could benefit all AML patients or only patients with a certain subtype of AML. It is hoped that patients with genetic
abnormalities indicating poor prognosis may respond to immunotherapy treatment. The use of immunotherapies may prove very important for elderly patients and those who do not qualify for allo-HSCT. In addition, immunotherapy may be an effective strategy to assist with full clearance of AML in patients in complete remission but with MRD positivity. It is hoped that immunotherapy may be able to eradicate LSCs to prevent relapse.

It is also important to consider potential mechanisms of resistance to immunotherapies, both intrinsic resistance and acquired resistance [256]. In regards to treatment of melanoma and other solid tumours, despite significant clinical benefits, there are still many patients who fail to respond to treatment. For patients with a functioning immune system, tumour immunogenicity, governed by the specific genetic abnormalities, plays a critical role in response. In addition, expression levels of immune checkpoint molecules on immune cells may also be important in response to therapy. For example, preclinical observations indicate that there is a threshold of PD-1 expression on T cells, above which they fail to respond to anti-PD-1 therapy [257]. More research on this is needed to fully understand these mechanisms and how they may apply to AML patients.

Acquired resistance to immune checkpoint blockade may be mediated by the compensatory upregulation of other immune checkpoint molecules. For example, resistance to anti-CTLA4 has been shown to be mediated by upregulation of PD-L1 on melanoma cells [258, 259]. This observation supports the combined blockade of CTLA-4 and PD-1 [253].

In regards to acquired resistance, antigen down-regulation may present a significant challenge for the use of CAR-T cells in treatment of AML. Insights into this mechanism of immune escape can already be gained from ALL patients relapsing with CD19-negative ALL post-treatment with CD19 CAR-T cell therapy [260]. A significant challenge for CAR-T cells is also T cell persistence in the patient. As discussed above, this may be assisted by combining CAR-T cell therapy with additional immunotherapies such as immune checkpoint blockade and cytokine therapy.

In conclusion, immunotherapy has emerged at the forefront for the treatment of many cancers. AML presents a challenging target for immunotherapies as summarised in this review due to severe immunosuppression in patients and low immunogenicity of AML cells, however despite this, clinical trials are forging ahead. While AML patients have a relatively low mutational burden, the diversity of genetic abnormalities and their influence on prognosis may complicate the effectiveness of immunotherapies enhancing the anti-leukemic immune response. More research is needed to better understand the effect of genetic abnormalities on the immune response. This work will enable future clinical trials to be strategically designed and evaluated according to a patient’s specific genetic abnormalities, thus providing a precision approach to immunotherapies. This approach will likely provide the best efficacy, limit unnecessary side-effect exposure and provide an optimal pharmacoeconomic model to develop these exciting new agents in AML.
Table 1: Selected current clinical trials of immunotherapies in AML

<table>
<thead>
<tr>
<th>Title of clinical trial</th>
<th>Phase</th>
<th>Identifier</th>
<th>Date commenced</th>
<th>Location</th>
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<td>Treatment of APL patients with ATRA, arsenic trioxide and Gemtuzumab Ozogamicin (GO) (anti-CD33 monoclonal antibody)</td>
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<td>Peginterferon Alfa-2a to Enhance Anti-leukemic Responses After Allogeneic Transplantation in Acute Myeloid Leukemia</td>
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<td>Trial of Daily Pulse Interleukin-2 With Famotidine in Acute Myelogenous Leukemia</td>
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Conflict of interest

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Associate Prof. Steven W. Lane: Assoc. Prof. Steven Lane is a clinical haematologist at the Royal Brisbane and Women’s Hospital, and team head at QIMR Berghofer Medical Research Institute. His research interests revolve around understanding the biology of leukaemia stem cell populations and leveraging their dependencies to find new treatments for patients with blood cancers. He is Associate Professor at the University of QLD Medical School.
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