Natural Killer Cell Cellular–Based Therapeutic Options for Management of Acute Myeloid Leukemia: Prospect and Challenges

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Abstract

Over the past decade, significant progress has been made in the management of acute myeloid leukaemia (AML). However, refractory disease and relapse remain major issues. These necessitate the need for novel therapeutic options to help achieve deeper remission and treat refractory and relapsed diseases for improved survival. Natural killer (NK) cell cellular therapies have been muted as an option. NK cells are a specialized population of innate lymphoid cells that possess constitutive capabilities against viral infections and cancer cells. Unlike T cells, NK cells do not need prior antigen sensitization to kill their target cells, thus their potential as immunotherapeutic agents. However, NK cells are noted to be dysfunctional in patients with haematological malignancies. Revitalizing them is another immunotherapeutic strategy. In this review, we summarize the biology of NK cells and the various forms of NK cell cellular therapies for the potential management of AML, both in preclinical studies and clinical trials.

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Introduction

Acute myeloid leukaemia (AML) is a clinical and genetic heterogeneous clonal, malignant disease of hematopoietic tissues that is characterized by the accumulation of abnormal (leukemic) blast cells, principally in the marrow, and

impairment in the production of normal blood cells. Until recently, there have been limited treatment options for AML which is often cytotoxic chemotherapy. However, since 2017, midostaurin became the first targeted therapy for the treatment of AML in about four decades[1], and with subsequent new approvals afterwards[2]. With an increasing lifespan worldwide, there is an increase in the incidence of AML. According to SEER statistics, In the United States alone in 2022, there will be an estimated 20,050 new cases of AML diagnosed representing 1% of all new cancer cases and 11,540 deaths representing 1.9% of all cancer deaths[3]. This showed an approximately 6% increase in the number of deaths from the SEER data from 2019. However, despite the progress made in drug therapy, relapse remains a major problem. However, haematopoietic stem cell transplant (HSCT) perhaps remains the only curative therapy for AML at the moment with its attending risk and side effects. Thus, newer therapeutic options are needed to achieve a greater complete remission (CR) with negative minimal residual disease (MRD). One such option is immunotherapy[4][5].

Immunotherapy in cancer is a type of treatment that harnesses the specificity and killing mechanisms of the immune system to target and eradicate malignant cells. Immunotherapy has been noted as a viable treatment strategy in the management of various cancers and was voted “breakthrough of the year” by Science in 2013[6]. Immune cells and biochemicals of the immune system are constitutively made to fight disease-causing microbes as well as their infected cells, and cells with potentials for neoplastic transformation. However, malignant cells have devised various means of evading immune cells through the loss of immunogenicity, upregulation of negative regulatory pathways or through the creation of an immunosuppressive microenvironment thereby making immune cells less potent in destroying cancer cells[7][8]. Extensive research in cancer immunotherapy and the dynamic interactions between cancer cells and host immune cells have brought up innovative ways of boosting the host immune cells or initiating novel ways to elicit immune responses in fighting cancer which has led to the approval of new therapies against both solid and hematological cancers[9][10]. Recently, Natural killer (NK) cell cellular-based therapies have been muted as one of the novel strategies in fighting cancers, especially haematological cancers. It has been observed that there is both a quantitative and qualitative dysfunction in NK cells in haematological cancers, and there is further impairment in their numbers and function as a result of chemotherapy, and radiation used during treatment[11][12]. Restoration of these immune impairments can improve therapeutic outcomes. Over the years, a better understanding of NK cell immunobiology coupled with improvements in molecular biology techniques have led to increased development in the field of NK cell cellular-based therapy in haematological cancers including chimeric antigen receptor (CAR)--modified NK cells[13][14][15][16][17], adoptive cell transfer[18][19][20], cytokines[21][22], bispecific natural killer cell engager (BiKE)[23][24][25], drug treatment[26][27][28]etc. Despite the major developments in NK cell-based therapies especially in AML, it is yet to make inroads into the clinics. This is a result of different factors, the chief one being clinical trials are still in progress in many of them. Herein, in this work, I look into the biology of NK cells, the various NK cell-based therapies being developed both in preclinical and clinical trials, and the challenges faced getting them to the clinic.

NK cell biology

NK cells are a distinct group of innate lymphoid cells that possess the intrinsic abilities to identify and eliminate virally infected cells and tumour cells. NK cells can be subdivided into various subsets depending on the relative expression of

CD56 ([neural cell adhesion molecule (NCAM)) and CD16. Natural killer cells comprise between 5-20% of circulating lymphocytes in humans[29]. NK cells are either CD56bright or CD56dim. The CD56dim set makes up about 90% of all peripheral NK cells, and represent the final stage of NK cell maturation with its attendant expression of maturity-related inhibitory receptors (killer cell immunoglobulin-like receptors [KIRs]), cytotoxic effector proteins (perforin and granzyme B) when at rest, and also high surface levels of CD16 (FcγRIIIa) that play a role in antibody-opsonized targets. The remaining 10% of the NK cell population are CD56bright and express lower levels of cytotoxic effector proteins (perforin and granzyme B) when at rest; also express dim levels of CD16. Unlike the CD56dim subset of NK cell that express KIRs as an inhibitory molecule, the CD56bright NK cells express CD94/NKG2A, CD94/NKG2C, and NKG2D receptors and possess distinct chemokine and homing receptor repertoires such as CCR7 compared with the CD56dim subset[30]. The CD56bright subsets are known potent producers of immunoregulatory cytokines such as interferon (IFN)-γ, tumour necrosis factor (TNF)-α/β and interleukin (IL)-10. This happens following combined cytokine receptor stimulation[31][32][33]. Traditionally, CD56bright NK cells display minimal antitumor responsiveness at rest, unlike the CD56dim subset that is known to exert strong cytolytic activity. Thus, CD56bright NK cells are considered ineffective for antitumour responses. However, CD56bright cells from multiple myeloma (MM) patients have been shown to display enhanced ex vivo functional responses when primed with interleukin 15[34][35]. NK cells are known to mature in the bone marrow (BM) and other secondary lymphoid tissues. However, the BM seems to be the primary site of NK precursor cells and other secondary sites such as the spleen and thymus do not deter NK cell growth and function[36][37][38], unlike T cells[37][39], however, later stages of NK cell development take place in the secondary lymphoid tissues (SLTs) such as the liver, lymph nodes and tonsils[40][41]. In fact, in the parafollicular T cell region of the lymph node that is rich in CD56 bright NK cells which differentiate into mature CD56dim NK cells after stimulation by interleukin 2[42]. The Lin−CD34+CD133+CD244+ HSCs are known to differentiate into CD45RA+ lymphoid-primed multipotential progenitor (LMPP) in the early stage of development which are found to also be CD38- and CD10-[43], but have CD62L[44]. The LMPPs have multiple lymphoid lineage potentials along with some residual myeloid potential, but lack erythroid and megakaryocytoid potentials; and no self-renewal capacity[45]. The LMPP transit into the common lymphoid progenitor (CLP) which lack potential for myeloid differentiation, but can make lineage commitment into all subset of lymphocytes i.e. Pro-B, Pre-T, NKPs, or other innate lymphoid cells (ILCs) [46]. CLP were earlier thought to be Lin− cKit^{low}Sca-1^{low}CD127^{hi} (IL-7Rα^{hi}) but were later refined to include high expression of Fms-related tyrosine kinase 3 (Flt3)[47]. CLPs have also been discovered to be associated with the expression of Ly6D, a surface marker that divides CLPs into two distinct populations. The Ly6d− subset of CLP called all lymphoid progenitor (ALP) has T and NK potentials, while the Ly6d+ subset, called BLP (B-cell-biased lymphoid progenitor), up-regulates the B-cell-specifying factors Ebf1 and Pax5, thus acting as B cell progenitors[48]. It should noted be that NK cells were for some time the only known innate lymphoid cells - a group of innate lymphocytes that do not express RAG-dependent rearranged antigen-specific cell surface receptors, until the discovery of lymphoid tissue-inducer (LTi) cells in the 1990s and helper-like innate lymphoid lineages from 2008. ILCs are classified into five groups, and this is based on their developmental course and cytokine profile. They include the cytotoxic NK cell, LTi cells which express the integrin α4β7, lymphotoxin (LT)α1β2, and lymphoid cytokine receptors, and helper-like ILCs (ILC1, ILC2 and ILC3) with their distinct functional expression like CD4+ T helper (Th) type 1, Th2 and Th17 cells[49][50][51][52]. The families of innate lymphocytes share a common progenitor known as the early innate lymphoid progenitor (EILP); the cytokine-producing

ILCs also have a more restricted progenitor known as common helper-like innate lymphoid cell progenitor (CHILP)[53][54][55]. All ILCs except NK cells require GATA-3 for their differentiation[56]. In addition, NK cells and ILC1 cells depend on two evolutionary-related T-box transcription factors (TFs) which are eomesodermin (EOMES) and T-box expressed in T cells (T-bet) for their development, though EOMES is strictly required for the development of NK cells, while ILC cells do not develop in the absence of T-bet in conjunction with Aiolos and Bcl6[55][57][58][59]. GATA-3, B-cell lymphoma/leukaemia 11B (BCL11B) and RORα are required for the development of ILC2 cells as well as the control of the production of type 2 effector cytokines, IL-5, IL-13 and IL-4[60][61]. The group 3 ILC cells are dependent on GATA-3, RAR-related orphan nuclear receptor γt (RORγt) and HIF1 for their development and production of cytokines IL-17 and IL-22[59][62]. In all these, mature ILCs can be generated from CLPs[55].

While the ontogeny of NK cells is not well understood, NK cell development has so far been classified into six stages. CLP in NK development transitions to CD7+, CD127+ (IL-7Rα+), CD122+ (IL-2Rβ+), CD117+ (c-Kit+), and IL-1R1low NK progenitors (NKPs) in the stage 1. The acquisition of CD122 shows a commitment to NK lineage development, promoting NK cell differentiation, functional maturation, and survival [63][64][65]. CD3ε–CD7+CD127+ cells characterize stage 2a of pre-NK cells, while the expression of IL-1R, a receptor for IL-1β differentiates stage 2a from Stage 2b[66]. The expression of activating receptors CD335 (Natural cytotoxicity receptor, NCR1, NKp46), CD337 (NCR3, NKp30) and CD161 marks the transition of NK cells from Stage 2b Pre-NK cells to Stage 3 immature NK cells (iNK) cells[67]. NKG2D uses DAP10 adaptor protein as an adaptor complex, while NCR1 uses CD3ζ and FcεRγ, and NCR3 CD3ζ only. Stage 4 of NK cell development is divided into two stages 4a and 4b, with the latter distinct from the former by the expression of NKp80. The stage 4a is NKp80 negative, and characterized by the expression of maximal levels of NKG2D, CD335, CD337, inhibitory NKG2A [CD159a, contains two immunoreceptor-based tyrosine inhibitory motifs (ITIMs)] and CD161 (NK1.1, KLRB1, NKR-P1A) which are CD56bright. At Stage 4b, NK cells become positive for NKP80 but still maintain their CD56bright status. Stage 5 is characterized by the down-regulation of CD56bright expression to CD56dim which are mature NK cells with the gradual up-regulation of CD94/NKG2C and CD16(FcγRIII), and down-regulation of CD56, c-Kit(CD117), and CD94/NKG2A[68]. The CD56bright NK cells are considered to be less mature than the CD56dim NK cells, thus, they reside primarily in SLTs, unlike the CD56dim that form the majority of NK cells in circulation[12][69]. Ultimately, the terminal maturation of CD56dim NK cells is identified with the expression of CD57 (HNK-1, Leu-7), and killer cell immunoglobulin-like receptors (KIR+/CD158+) which is the stage 6 of NK cell development[70][71][72] (Figure 1).

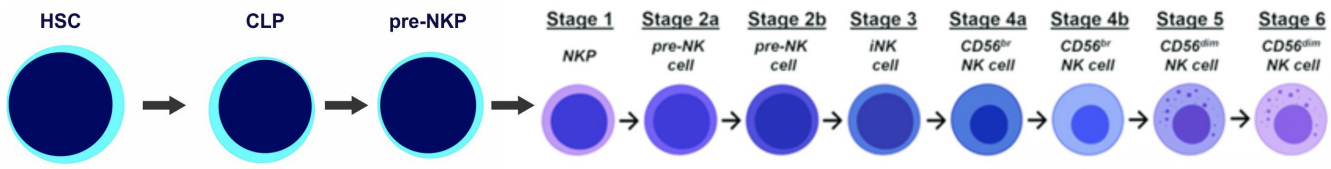

Figure 1. The schematic diagram of NK cell development from the haematopoietic stem cell (HSC) to the terminal stage 6.

NK cells recognition of self from non-self

The process of NK recognition of self from non-self is still being debated. This education process is as a result of NK cells

interaction with self-major histocompatibility complex (MHC)-I[73]. Several studies examining the basis of tolerance in NK cells have linked it to MHC-I surface expression. The killer immunoglobulin-like receptor (KIR) family of receptors is the NK group of receptors that primarily associate with MHC-1, and through them, MHC-I regulates NK cell function. In trying to explain the NK cell education process, Yokoyama and colleagues proposed the NK cell licensing hypothesis, which states that an NK cell must engage self-MHC class I to be responsive to subsequent stimuli received by activating receptors, which they termed "licensing." Conversely, those NK cells that failed to engage in self-MHC class I were considered "unlicensed"[74][75]. Thus, this process gives rise to two types of self-tolerant NK cells: (a) the licensed NK cells, which maintain self-tolerance by direct inhibition by binding to self-MHC and (b) the unlicensed NK cells, which cannot engage self-MHC, but are self-tolerant due to their inherent resistance to stimulation received through activating receptors. Later on, Raulet and Vance their model of NK cell self-tolerance was termed the arming and disarming model. According to the arming model of NK cell education, the KIR inhibitory receptor interaction with MHC class I molecules give rise to inhibitory signals that go on to promote functional maturation of human precursor NK cells but not mature NK cells. This hypothesis appears counterintuitive in that these receptors are essentially inhibitory. However, signalling through these receptors may seem more complicated than previously thought. The disarming model, on the other hand, proposes that both precursor and mature NK cells that lack self-MHC-I inhibitory receptors are rendered hyporesponsive upon receiving sustained positive signalling via activating receptors[76]. Thus, these models show that increased expression of inhibitory receptor signalling in comparison to activating signalling invariably leads to a heightened response of NK cells; therefore, NK cells with functional copies of KIR genes are functionally more competent than those without in their education process [77]. As a result of the alterations in the expression of inhibitory receptors during NK cell development, various combinations of inhibitory receptors can be expressed on distinct NK cells especially in a disease state, thus, making it function like a rheostat to set a quantitative threshold of NK cell responsiveness during the education process[78]. This is the rheostat model which incorporates concepts from the licensing and disarming model that different inhibitory receptors can bind MHC ligands with varying affinities; and the interactions between the various inhibitory receptors and the expressed MHC molecules will result in varying degrees of inhibition between distinct NK cells which allows for a range of NK cells responses[79][80]. Just like the diversity of the MHC molecules, the KIR displays a high level of polymorphism. The KIR haplotypes are grouped into two primary sets, known as “A” and “B”[81]. The KIR A haplotypes mainly contain inhibitory KIR genes and only one activating KIR gene KIR2DS4. On the other hand, KIR B haplotypes have different numbers and combinations of activating KIR genes besides inhibitory KIR genes.

NK cell signalling and effector functions

NK cells do not express clonotypic receptors. However, they are still able to generate significant anti-tumour cytotoxicity as well as the production of inflammatory cytokines. These actions are believed to be controlled by an array of germline-encoded activating and inhibitory receptors which include NKG2D, NCR1, NCR2, NCR3, NKG2C, CD244, Ly49D, Ly49H, KIRs, CD94/NKG2A and leukocyte Ig-like receptor 1 (LIRl). Their receptors are transmembrane proteins which have an extracellular ligand-binding portion and an intracellular cytoplasmic tail. The intracellular cytoplasmic tail of the inhibitory receptors contains immunoreceptor tyrosine-based inhibition motifs (ITIMs) that can directly stimulate their protein

phosphatases. If on the other hand, it is an activating receptor which lacks signaling domains in their cytoplasmic tails it will indirectly stimulate protein kinases by recruiting adaptor proteins containing immunoreceptor tyrosine-based activation motifs. The adaptor molecules that propagate the activation receptor signalling include FcεRIγ, CD3ζ, and DAP12. NKG2D and Ly49H can also propagate signals through the YINM motif present within the adaptor, DAP10. The activating receptor NKG2D is a type II transmembrane and C-type lectin-like type II homodimeric receptor that is involved in NK cell lysis [just like other activating receptors, (NCR) NKp46 (NCR1), NKp30 (NCR3), and NKp44 (NCR2)]. It is constitutively expressed in NK cells, and its signalling is mediated through the adapter proteins DAP10 and DAP12 via YINM and ITAM tyrosine-based signalling motifs- DAP10 is involved in the recruitment and activation of the p85α subunit of PI(3) K and Grb2, while DAP12 is involved in the recruitment of ZAP70 and Syk to initiate NKG2D-mediated NK cell activation. In addition to these activating receptors, activating co-receptors such as 2B4, NTB-A, DNAM-1, CD59, and NKp80 play a complementary and synergistic role in NK cell activation. 2B4 and NTB-A are part of the signalling lymphocyte activation molecule (SLAM) family that aids the potentiation of NK cell cytotoxic activity by the main triggering receptors[82][83]. 2B4 and NTB-A are associated with signaling lymphocyte activation molecule (SLAM)-associated protein (SAP), a molecule involved severe form of immunodeficiency, the X-linked lymphoproliferative syndrome type 1 (XLP-1). The 2 two co-receptors have been noted in the absence of SAP to deliver inhibitory, instead of activating signals[83][84][85]. CD59, a glycosylphosphatidylinositol (GPI)-linked protein, and a PNH marker depends on the simultaneous engagement of NKp46 and NKp30 receptors via the tyrosine phosphorylation of CD3zeta chains to enhance NK cell-mediated cytotoxic activity[86]. Low CD59 is associated with increased proliferation and abnormal coagulation function in AML[87]. The DNAX Accessory Molecule (DNAM-1 or CD226) is an adhesion molecule that is involved in NK cell activation. DNAM-1 has two ligands, namely poliovirus receptor (PVR) and Nectin-2 that are widely expressed in hematological cancers[88]. The dual interaction of the ligands with the activating coreceptor, DNAM-1 and the inhibitory receptors CD96 and TIGIT (DNAM-1 enhances NK cell-mediated cytotoxicity via PVR and Nectin-2, whereas, TIGIT recognition of these ligands exerts an inhibitory effect on NK cells by diminishing IFN-γ production, as well as NK cell-mediated cytotoxicity) makes them an ideal target for immunomodulation in cancer[89][90][91]. Studies have shown there is a reduced expression of DNAM-1 in AML, while the inhibitory receptors TIGIT and TIM-3 are increased[92]. Consequently, loss of DNAM-1 and reduced expression of PVR is a major NK cell escape mechanism in AML[93].

The inhibitory receptors KIRs and CD94/NKG2A recognize HLA class I and non-classical MHC class Ib molecules such as HLA-E respectively. KIRs are clonally distributed and only a fraction of NK cells express a given KIR making them highly polymorphic. The best-characterized ligands for KIR are HLA class I molecules that express either the Bw4, C1 or C2 motif. KIR2DL1 recognizes HLA-C alleles characterized by Lys at position 80 (HLA-CLys80), KIR2DL2/3 recognize HLA-C alleles characterized by Asn at position 80 (HLA-CAsn80), KIR3DL1 is specific for HLA-B alleles sharing the Bw4 supertypes specificity (HLA-BBw4), and KIR3DL2 recognizes HLA-A3 and -A11 alleles[88]. The KIR system acts through specific interactions and varying degrees of signal strength to diversify NK cell stimulation. Thus, weakly inhibitory KIR/HLA combinations permit a lower threshold for cell activation and vice versa. Therefore, when there are no effective inhibitory interactions, target cells are susceptible to NK-mediated killing. A study by Dai et al showed that increased KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1 and KIR3DL2 mRNA levels were significantly related to poor prognosis and overall survival (OS) in AML patients[94] (Figure 2).

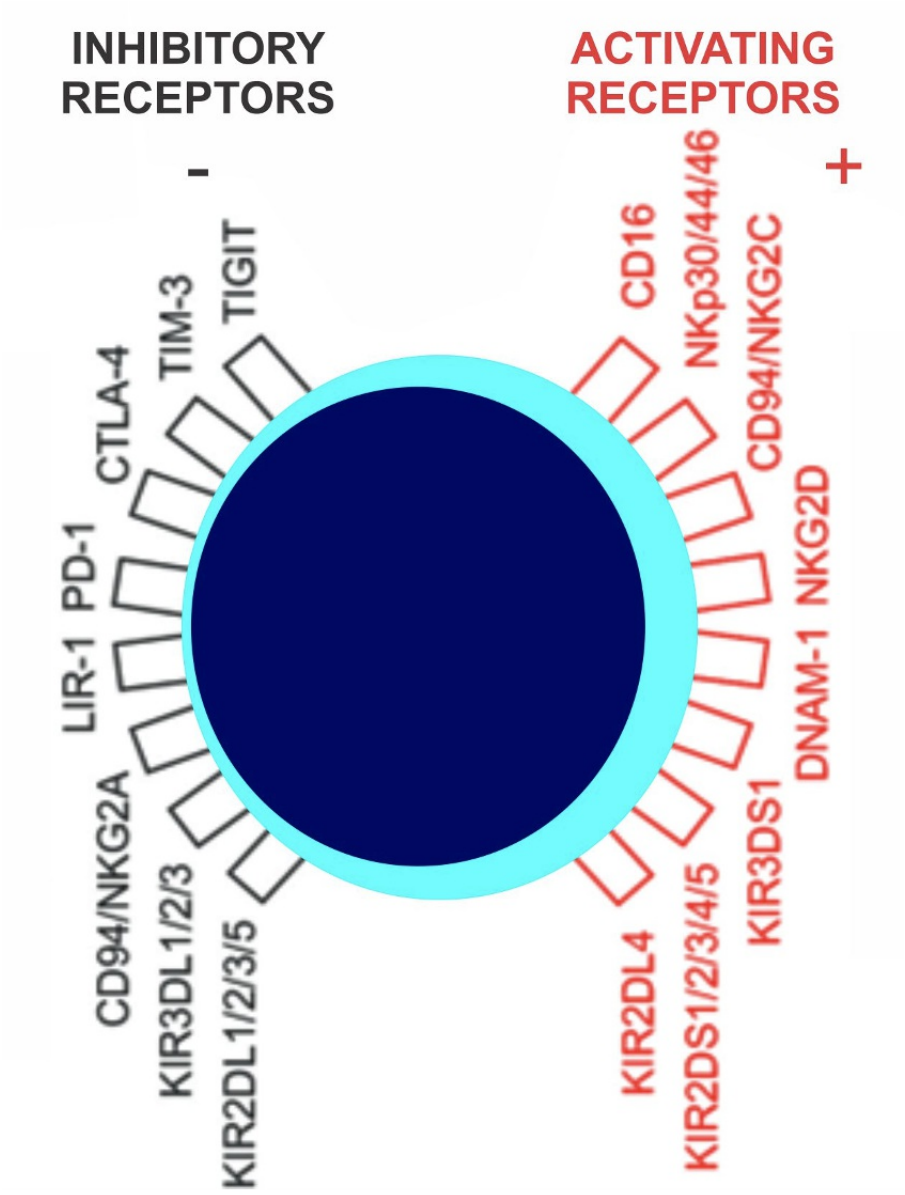

Figure 2. A schematic representation of NK cell activation and inhibitory receptors.

In performing their effector functions i.e. cytotoxic death of target, NK cells are reported to use various mechanisms. This requires certain processes. In the process of target destruction, NK cell first recognizes their target through certain molecular mechanisms via inhibitory receptors that recognize surface molecules expressed at steady state and via activating receptors that recognize stress-induced molecules. Once a target cell is recognized, there is a direct interaction of NK cell with the target through the formation of an immunological synapse which facilitates target cell death through some mechanisms. The predominant mechanism by which human NK cells kill their target is by the release of lysosomal-related organelles known as lytic granules, in a process referred to as 'degranulation'[95]. These lytic granules are delivered to the target cell through membrane fusion at the immunological synapse, a process that involves cytoskeletal rearrangement which includes actin polymerization and polarization of the cytoskeletal rearrangement-assisted

microtubule-organizing centre (MTOC) towards the target cell[96][97][98][99]. These lytic granules once polarized move along the microtubules and at the immunological synapse fuse with the membrane of the target cell and release their lytic enzymes which cause the activation of an apoptotic process within the target cell[100]. The major components of the lytic granules in the “degranulation” process are Granzyme B and perforin. Perforin, a 60-70-kDa pore-forming glycoprotein forms pores in target cells leading to osmotic lysis. A partial deficiency in perforin production causes increased susceptibility to haematological cancers[101]. On the other hand, Granzyme B, a class of serine proteases can induce apoptotic cell death through caspase-dependent and independent mechanisms.

Another mechanism of NK cell-induced target cell death is the engagement of death receptors (DR) through their cognate ligands which are present on the surface of target cells. The TNF-related apoptosis-inducing ligand-receptor (TRAIL-R) and Fas (CD95) are two of the death receptors present on target cells and are activated by their cognate ligands, Fas ligand (FasL) (CD95L) and TRAIL, which are found on NK cells. The binding of the DR by their cognate ligands induces a conformational change in the receptor by their oligomerization and recruitment of adapter proteins which will initiate the process of apoptosis either directly via effector caspases or indirectly via the intrinsic mitochondrial pathway[102][103].

In addition to their cytotoxicity, NK cells are also known to be potent producers of pro-inflammatory and immunosuppressive cytokines. These actions are mediated by $CD56^{bright}$ NK cells, making them less cytolytic[31][32]. The two prominent cytokines produced are IFN-γ and TNF-α, and depending on the inflammatory environment IL-5, IL-10, IL-13, some growth factors like IL-3, G-CSF, GM-CSF. NK cells also produce chemokines like CCL1, CCL2/MCP-1, CCL3 (MIP-1α), CCl4 (MIP-1β), CCL5 (RANTES), XCL1 (lymphotoxin), CXCL10/IP-10 and CXCL8 (IL-8) which can attract effector lymphocytes and myeloid cells to inflamed tissues[104][105][106]. Several transcriptional regulators are involved in the production of these inflammatory cytokines. These include the c-Fos and c-Jun heterodimer of the AP-1 TF genes, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and nuclear factor of activated T cells[107][108][109][110]. The NK cell cytokine secretion can be both beneficial and deleterious. NK cells have been reported to display “split anergy”, a process that exhibits increased cytokine secretion, but with loss of cytotoxicity as a result of interaction with cancer stem cells[105]. This is an IFN-γ-mediated process. However, some of these cytokines also mediate some beneficial immunoregulatory functions. IFN-γ plays a role in dendritic cell maturation and indirectly promotes adaptive T-cell responses. It also aids in the activation of helper T cells to a Th1 phenotype[111]. TNF-α, in a similar fashion, is involved in B cell proliferation and its anti-proliferative effect against tumour cells. TNF-α also mediates endothelial activation with increased production of adhesion molecules and inflammatory cytokines.

In addition to its MHC-I targeting of diseased cells, NK cell is also involved in antibody-dependent cellular cytotoxicity. NK cells do have Fc receptors that can bind antibodies in their Fc region. They bind to the Fc portion of immunoglobulins through their own FcγRIIC/CD32c and FcγRIIIA/CD16a[112][113]. FcγRIIC has an ITAM in its cytoplasmic tail just as FcεRI-γ chains or CD3-ζ chains within the cell membrane equally have, though the primary activating receptor is the low-affinity FcγRIIIA/CD16a that binds the Fc domain of IgG. However, upon binding to FcγR there is phosphorylation of the ITAMs which initiates a signal cascade i.e. there is binding to tyrosine kinases ZAP-70 and Syk, with subsequent activation of the PI3K, NF-κb and ERK pathways that cause NK cell degranulation, cytokine release, and tumour cell lysis[114][115][116].

Although NK cells do not have clonotypic receptors like T cells, studies have shown that a relatively small population of them can elicit memory-like responses[117][118]. Memory NK cells were first described in mice deficient in T & B cells. Following secondary exposure to specific haptens such as 2,4-dinitrofluorobenzene and oxazolone, NK cells were able to mediate contact hypersensitivity response against the haptens and the adoptive transfer of previously sensitized NK cells persisted about for 4 weeks[119]. The development of memory NK cells has been studied in mice infected with murine cytomegalovirus (MCMV). The C57BL/6 mice due to their expression of the activating receptor Ly49H which is specific for the viral glycoprotein m157 expressed on virally infected cells were an ideal choice to study memory NK cells. Following MCMV infection it was observed that a small population of NK cells persists despite NK cell contraction. Upon isolation and adoptive transfer to naïve neonate mice which lack effective MCMV defence, these memory NK cells are better able to protect and prevent MCMV-mediated death than NK cells isolated from naïve hosts[117][120]. More research has shown that cytokine-mediated activation particularly IL12 and IL18 can induce NK cells with such memory traits, and when adoptively transferred back into mice led to heightened IFN-γ secretion for some weeks along with cytotoxicity usually observed in resting NK cells[121][122]. Similarly, Jin et al showed that the in vivo pre-activation and re-stimulation of NK cells with interleukins (IL-12, IL-15, and IL-18) led to enhanced IFN-γ secretion which could be transferred to the next generation of NK cells and was associated with prolonged survival. The increased IFN-γ secretion was suggested to be likely NKG2D-dependent[123]. Also, Brillantes and Beaulieu have shown that NK cells can produce memory and memory-like responses towards different microbial pathogens[124]. The ability of these memory NK cells to produce enhanced levels of IFN-γ with cytotoxic granules and their ability to persist for a long time, these cells are being muted as potential cancer chemotherapies[125][126][127]. It remains to see how they can be harnessed.

NK Cell Dysfunctions in AML

From its biology, NK cells can eliminate malignant cells by exerting both direct and indirect anti-neoplastic effects through their cytotoxic and immunoregulatory functions which are important to direct an enhanced immune response against cancer cells. However, studies have shown that in AML, the immune microenvironment is impaired including myeloid and erythroid differentiation, macrophages and T-cell functions, osteogenesis as well and NK cell immune surveillance[128][129][130][131][132][133]. Guo et al using single-cell RNA sequencing observed significant differences between normal and AML BM immune cells[134]. Kutznesova et al also reported impaired degranulation of NK cells in ex vivo AML models with increased transcriptional signatures observed in IL-6-STAT3 and IL-1β/TNFα[135]. Thus, impaired immune function especially in NK cells is one of the means that AML escape immune surveillance. AML evade NK cell immune surveillance through different ways including 1) the reduction in the number of natural cytotoxicity receptors (NCRs) on NK cell surface, 2) the overexpression of the inhibitory receptors KIRs and NKG2A with the resultant increase in inhibition of cytotoxicity, 3) the impaired maturation of NK cells with majority of cells expressing CD56bright/dim KIRs-CD57- 4) the expression of checkpoint inhibitors like PD-1 and TIGIT resulting in cells with reduced proliferative potential and lower cytotoxic and cytokine-producing capabilities.

One of the features of AML progression is the reduction in the number of functionally active NK cells. There is an inverse

correlation between the anti-leukaemic activity of NK cells and disease progression in AML with the observed suppression of NK cell number during active disease, increase in number in remission, and suppression again in the event of a relapse[136][137]. Conversely, NK cell fusion post-HSCT was associated with reduced relapse and without an increased incidence of graft-versus-host-disease; in a study, the 1-year OS, CR rate, ORR, relapse rate (RR), acute and chronic GVHD rates were 69%, 42%, 77%, 28%, 24.9% and 3.7%, respectively[138][139][140]. In addition, NK cells have a significant correlation to OS and risk stratification in AML patients[141].

The NCRs are surface receptors that are important in NK cell cytotoxicity. Blocking of these receptors inhibits NK cell cytotoxicity. NCR expression on NK cells is either bright (NCR^{bright}) or dull (NCR^{dull}) and most healthy individuals express NCR^{bright} phenotype. Studies have shown that there is a correlation between NCR expression and NK cell-mediated cytotoxicity[142][143]. In AML, these receptors are also under-expressed which affects NK cell cytotoxicity and cytokine production[144][145]. In addition to the low display of NCRs by NK cells in AML, there is also a defective expression of ligands for NK cell activating/inhibitory receptors by AML cells[133][146][147]. This lack of expression of NCR ligands on their cell surface makes it difficult for NK cells to target them via NCR engagement allowing them to escape immune surveillance. For example, the activating receptor found on NK cells, NKG2D interact with its ligands (NKG2DL) which comprise two members of the MHC class I-related chain (MIC) family (MICA, MICB) and six members of the UL16-binding protein (ULBP) family of proteins (ULBP1-6) and are generally not found on healthy cells but are induced on the surface of malignant cells. The NKG2D/NKG2D-L system has been observed as an important player in tumour development generally in cancer patients. The expression of some of these NKG2DL is regulated by c-myc and DNA methylation, making them therapeutic targets for NK cell therapy[148][149][150][151]. Furthermore, tumour cells cause the proteolytic shedding by metalloproteases and release of soluble NKG2D-L causing downregulation of NKG2D as well as block receptor activation[152]. In their investigation of soluble NKG2DL in 205 leukaemia patients, Hilpert et al discovered that about 75% of them expressed at least one NKG2DL at the surface, and all investigated patients had elevated soluble NKG2DL levels in their sera. They also demonstrated that soluble NKG2DL in their sera reduced NKG2D expression in NK cells which resulted in impaired antileukaemic activity[153]. The clinical importance of the NKG2D/NKG2D-L system is also highlighted in a study that shows that the blocking of MICA/MICB shedding prevented cancer cell growth in immunocompetent mouse models and with the reduction of melanoma metastasis in a humanized model[154]. In contrast, DNAM-1 and its ligands (CD112, 155) are frequently expressed in leukaemic blasts, and its expression is associated with a favourable prognosis[145], [155][156]. However, under-expression of DNAM-1 has also been reported in AML, and it correlates with poor NK cell lysis[147][157][158].

Alterations in the expression of inhibitory receptors have also been described in AML. In their research, Sandoval-Barrego et al reported that patients with all FAB types of AML had overexpression of inhibitory receptors CD158b and NKG2A and decreased expression of the activating receptor NKp46[159]. The CD94/NK group 2 member A (NKG2A) heterodimeric receptor which binds to the non-classical HLA-E on cancer cells is one of the most prominent NK inhibitory receptors. NKG2A levels have been observed to be higher in the peripheral blood NK cells of patients with AML when compared with NK cells of age-matched controls[160]. Its ligand, HLA-E is known to be overexpressed in several cancer types, and it is also associated with poorer prognosis[160][161]. Administration of a novel anti-human NKG2A antibody was able to impede

tumour cell growth in leukaemic cells suggesting HLA-E could be a therapeutic target[162]. The KIR inhibitory receptors have also been studied. Shen et al reported that inhibitory KIR ligands were present in significantly higher frequencies in the prognostically poor risk group compared to those with favourable risk. Ghasemimehr et al in their research of gene expression of activating and inhibitory receptors of NK cells in patients with newly diagnosed AML before and after induction therapy reported a 6-fold increase in KIR2DL1 expression compared to healthy controls and a significant decrease in mRNA expressions of KIR2DL1 and NKG2A after induction therapy[163]. Yang et al also reported that the levels of other inhibitory receptors like TIM-3, ILT-4, ILT-5, and PD-1 were increased in NK cells from patients with AML; Siglec-7^{+} NK cells displayed greater cytotoxicity in AML patients[164].

Defective maturation of NK cells

The process of NK cell development involves different stages that are regulated by cytokines and transcription factors. The process earlier described, moves them from the precursor stage through different maturation phases until they acquire full maturation with the expression of a host of receptors, especially the NKG2A or KIRs. This process transforms the NK cell into a cell with a high cytotoxic capacity having the ability to recognize and eliminate cancer cells and viruses. However, this process can be hijacked by AML. Mundy-Bosse et al in their study on AML cells evasion of NK cells using specific murine maturation markers showed that there was the selective loss of the intermediate (CD27+CD11b+) phenotype with the upregulation of the immature phenotype (CD27+CD11b–). The NK cells in AML also had lower levels of T-bet and EOMES along with the upregulation of microRNA miR-29b, a regulator of T-bet and EOMES indicating a block in NK cell differentiation by AML[165]. In their study on AML patients, Christen et al were able to delineate three different groups based on their NK cell maturation profile. This include: the hypomaturation (CD56bright/dim KIRs–CD57–), intermediate (CD56dim KIR–/+ CD57–/+) and hypermaturation (CD56dim KIRs+ CD57+) groups. They equally reported that patients in the hypomaturation group showed a poor 3-year overall survival and relapse-free survival, suggesting that maturation profiles of NK cells in AML may play an important role in prognostication and clinical course of the disease[166][167]. In their most recent work (NCT02320656), Chrieten et al were able to demonstrate the presence of a moderate to drastic accumulation of CD56–CD16+ unconventional NK cells that displayed decreased expression of NKG2A as well as the activating receptors NKp30 and NKp46 in about a quarter of AML patients studied. These NK cells were associated with a significantly decreased OS and EFS, and a poor clinical outcome[168]. Liu et al on their part showed the expression characteristics of antigens and functional markers of NK cells in AML patients; NK cells were divided into two groups: CD3-CD56^{high}CD16- (CD56^{high}) and CD3-CD56^{dim}CD16+ (CD56^{dim}). The expression of CD56high NK cells was higher in AML patients than in healthy controls, and DNAM-1 expression was significantly low in CD56high NK cells, while NKG2D, DNAM-1, and perforin were significantly low in CD56dim NK cells[169]. Single-cell profiling also revealed three subsets of NK cells in the bone marrow of AML patients which also showed stress-induced repression of NK cell effector functions. This also showed the role AML plays in NK maturation, and how it affects the course of the disease.

Immune checkpoint inhibitor expression

Immune checkpoint molecules are part of the arsenals of the immune system that play an important role in self-tolerance and the prevention of lysis of self-cells. Immune checkpoint molecules are expressed on many immune cells[170]. Some of the immune cells expressed in NK cells include PD-1, TIM-3, LAG-3, TIGIT and SIGLEC-7. Mature NK cells are known to express PD-1 when stimulated by MHC class I-deficient tumour cells or infected cells. These cells display reduced proliferative and cytolytic abilities along with lowered production of cytokines. Targeting immune checkpoints has been clinically proven and approved for the management of some cancers, and the inhibition of PD-1 interaction with its ligand PDL-1 have been shown to restore NK cytolytic activity in some cancers[171][172][173]. PDL-1 expression is elevated in AML patients, though its clinical significance to NK cell function is not well understood[174]. Elevated PD-L1 expression in AML is associated with poor OS rate[174][175][176].

Another immune checkpoint T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) originally described on T-cells is known to be expressed on the surface of NK cells, while its ligand Galectin-9 (Gal-9) is also expressed in AML blasts. TIM-3 is reported to be associated with disease activity in cancer. In AML, TIM-3 is reported to be associated with poor prognosis. However, there is contradictory evidence to this. Darwish et al. and Kamal et al. both reported TIM-3 as a marker of poor prognosis in AML, while Xu et al. and Rakova et al. reported it as a good prognostic marker[177][178][179][180]. High levels of soluble Gal-9 have been demonstrated in the serum of AML patients, and its interaction with TIM-3 on leukaemic stem cells leads to the activation of the NF-kB and β-catenin pathways which play a role in leukaemic cells' self-renewal[181].

NK cell-based immunotherapy in AML

Adoptive NK cell transfer

While T-cell immunotherapy has gained prominence and approval in the management of haematological cancers, the use of NK cells has slowly gained more attention most especially because of its safety profile. Moreover, alloreactivity of NK cells in the allo-HSCT setting which is triggered by a mismatch between the inhibitory receptors on the donor NK cells and the HLA class I molecules on recipient cells has been observed and muted as a therapeutic strategy, especially in the management of leukaemia[182][183]. This alloreactivity of NK cells in leukaemia is known to be mediated through the graft-vs-leukaemia effect, and it is also beneficial in the prevention of graft-vs-host disease (GvHD) by destroying the APCs of the recipient as well as fighting some infections. Ruggeri et al in their study of the impact of donor-versus-recipient NK cell alloreactivity on survival in acute leukaemia patients reported EFS at 5 years of 60% in the group with KIR ligand incompatibility against 5% in the group without KIR ligand incompatibility and KIR ligand incompatibility was the only independent predictor of survival in AML[184]. In a related study, Mancusi et al demonstrated the effect of KIR ligand–mismatched NK cell donors on acute leukaemia. They showed that in 69 patients that underwent HSCT with donor-vs-recipient NK-cell alloreactivity, transplantation from donors with KIR2DS1 and/or KIR3DS1 was associated with reduced risk of non-relapse mortality, superior EFS, and a 50% reduction in infection rate[185]. Taken together, the adoptive transfer of NK cells is seen as a viable option in the management of leukaemia.

Adoptive NK cell transfer can be done in the HSCT or non-HSCT setting, and at the same time it can be either autologous

or allogeneic[186][187]. As a therapeutic strategy, autologous NK cell adoptive transfer is based on the extraction of the patient's own NK cell from the peripheral blood which is then expanded ex vivo and transduced back to the patient. This has its advantages in terms of convenience of source of NK cells, independence from immunosuppressants, and low likelihood of GvHD. To generate sufficient and high-quality NK cells, cytokines such as IL-2, IL-12, IL-15, and IL-18 are used to stimulate NK cells to enhance their effector functions and proliferative capabilities. However, the increased proliferative capacity does not necessarily lead to a significant therapeutic outcome and this is due to the inhibitory effect of the patient's HLA ligands. In some cases, the quality of NK cells may be below par because of prior heavy pretreatment of patients giving rise to poor effector functions. While this may be so in AML, autologous adoptive NK cell transfer has shown efficacy in solid tumours and some haematological cancers[188][189]. Various strategies are being developed to restore NK cell function. Wang et al in their study noted that increased levels of TGF-β1 impaired bone marrow NK cells, and pharmacologic blockade of TGF-β1 using galunisertib or anti-TGF-β1 antibodies can restore NK cell effector functions[190]. Also, Lirilumab, an anti-KIR antibody which potentiates NK cells has been shown to enhance therapeutic response as a combination therapy in vitro and in vivo, however, in the EFFIKIR randomized double-blind 3-arm placebo-controlled trial (NCT01687387) it failed to improve leukaemia free survival in elderly AML patients[191][192][193]. These findings have caused a shift from autologous NK cells to allogeneic NK cell transfer researchers.

For allogeneic NK cell transfer, NK cells obtained from healthy, HLA-matched or haploidentical donors are prepared and expanded under standard conditions (Figure 3). The NK cells are derived from different sources just like in autologous transfer which include peripheral blood NK cells, umbilical cord blood NK cells, NK cell lines, and stem cell-derived NK[194]. Ruggeri et al in their study showed that allogeneic NK cell transfer in AML patients induced a significant EFS[184]. Passweg et al observed an increase in donor chimerism and a decrease in chimerism and relapse in one AML patient each[195]. Different clinical studies of allogeneic NK cell transfer in the HSCT setting have shown tolerability and good efficacy[196][197][198][199]. Several patients may not be eligible for HSCT, but this has not hindered the development of allogeneic NK transfer outside the HSCT setting. Miller et al performed allogeneic NK cell transfer outside the HSCT setting, and 5 out of 19 were able to achieve complete remission; this was significantly higher in those with KIR–ligand mismatched donors[200]. Modifications to their approach have been replicated in other studies[201]. These methods equally have their challenges which include, low clinical-grade activation, lack of in vivo persistence and problems with ex vivo expansion. In all, adoptive NK cell transfer appears as a sound therapeutic strategy for AML for both induction remission and maintaining CR.

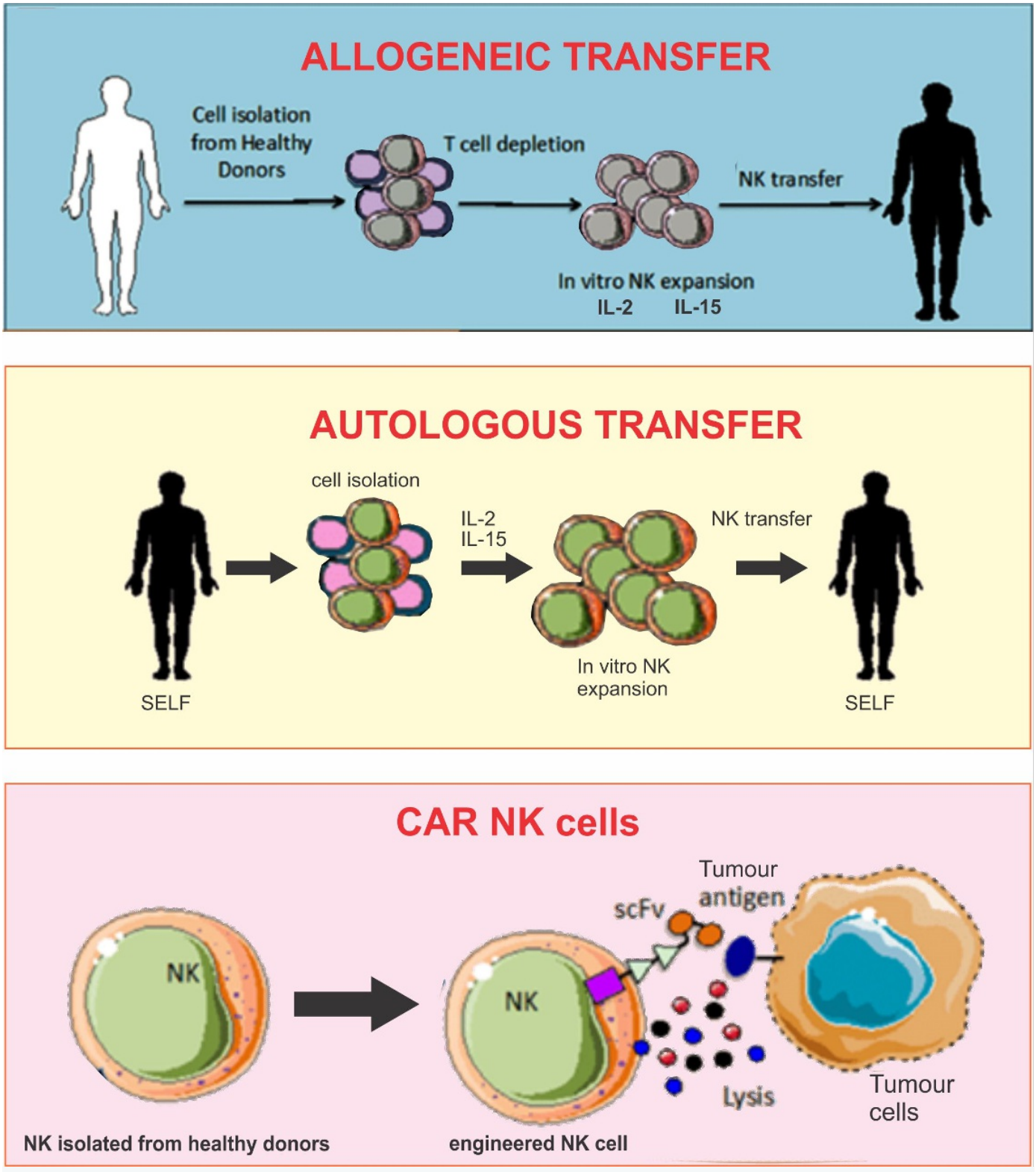

Figure 3. Schematic representation of adoptive NK cell transfer for AML patient. For the allogeneic NK cell transfer, NK cells are isolated from a healthy HLA-matched or haploidentical donor, and after T cell depletion and in vitro expansion, it is infused into the AML patient. For the autologous NK cell transfer, after NK cells are isolated from the peripheral blood of the AML patient, there shall be T cell depletion, followed by in vitro expansion before it is infused back into the patient. In CAR NK cell therapy, NK cells can be harvested from different sources which are then engineered to express specific receptors that recognize ligands on the AML cells leading to its destruction.

Chimeric antigen receptors (CAR) NK cell therapy

Following the success of CAR-T cell therapy in the management of B-cell precursor ALL and B-cell lymphoma, there have been a lot of optimism for cellular therapy in the management of other neoplasm including AML. Despite such optimism, CAR-T cell therapy is yet to become a reality in the management of AML due to adverse events like cytokine release syndrome (CRS)[202][203][204]. Other obstacles encountered with CAR-T cells include inefficiencies of T cell isolation, modification and expansion and high costs[205]. There is much enthusiasm that CAR-NK cells can prove a better alternative to CAR-T cells due to their shorter lifespan, favourable toxicity profile, and lower manufacturing costs[206]. Though it has some advantages, it is still yet to be translated into a treatment option. Some challenges are still faced including a loss of targeted antigen, hostile tumour microenvironment, and tumour heterogeneity. However, with progress made in NK cell engineering and target design, it is expected to prove efficacious in future trials. Liu et al, generated a CAR NK cell product from UCB NK cells that were transduced with a retroviral vector expressing genes that encode anti-CD19 CAR, interleukin-15, and inducible caspase 9 as a safety switch and were infused into 11 patients with B cell lymphoma and CLL in a phase 1/2 trial. Out of the 11 patients in the study, 7 had a CR, and 1 had remission of the Richter's transformation but with persistence of the CLL. Responses were seen within 30 days, the CAR-NK cells persisted for about 12 months, and there were no reported adverse events like cytokine release syndrome, neurotoxicity, or GvHD[207]. This reflects some optimism in AML management. In preclinical studies, allogeneic CAR.CD123-NK cells were able to induce significant anti-leukaemic activity both in vitro against CD123+ AML cell lines and CD123+ primary blasts and ex vivo in animal models[208]. Another study using CD33/FLT3 CAR-NK cells showed antileukaemic activity against primary AML blasts and LSC-enriched target cell populations as well as demonstrating improved survival in an MV4-11 xenograft AML mouse model[209].

CAR NK cell therapy has also shown efficacy in human clinical trials. In a first-in-human phase 1 clinical trial, Huang et al infused anti-CD33 CAR NK cells into 10 R/R AML patients after preconditioning with fludarabine and cyclophosphamide. 60% of the patients had a complete response 28 days after the infusion of the CAR-NK cells, and only one patient developed grade 2 CRS which was alleviated with dexamethasone[210]. There were no reported incidences of neurotoxicity or any other adverse events. A number of CAR-NK preclinical and clinical studies are underway across different cancers[211][212][213]. Unfortunately, the phase 1 NKG2D CAR NK cell therapy for R/R AML (NCT05247957) patients was prematurely terminated[214]. For now, CAR NK cell therapy is a prospective option in the management of AML.

Antibodies

The receptor-ligand interaction and antibody-dependent cell cytotoxicity are two ways that NK cells can be activated. Antibody mediation of these processes has thus been seen as a therapeutic strategy in AML. These are achieved either through tumour-associated antigens or targeting NK cell inhibitory receptors through antibodies.

Antibodies targeting tumour-associated antigens

The mechanism of this process is through the induction of ADCC by NK cells. While unconjugated antibodies have poor

efficacy when used alone, the engineering of antibodies' Fc parts can enhance their affinity to CD 16. Preclinical studies investigating these antibody-mediated actions have shown promising results. Riegg et al developed an anti-tumour antibody targeting CD133, a common protein on the surface of B-ALL cells which specifically activated NK cells that lysed the B-ALL cells[215]. This molecule was also used by Koerner et al in AML cells and xeno-transplanted mice and also cell lysis of CD133 bearing AML cells[216]. Steinbacher et al were also able to use Fc-optimized NKG2D-immunoglobulin G fusion proteins to activate NK cells against leukaemia cell lines (including AML) and primary AML cells with significant activity[217]. These works show that the use of Fc-optimized antibodies against specific antigens expressed on AML cells can be a viable option.

The antibody-drug conjugates (ADCs) and antibody-radio conjugates (ARCs) have been shown to be a good therapeutic strategy for enhancing the potency of antibodies. Gemtuzumab ozogamicin, a calicheamicin conjugate of anti-CD33 antibody is approved for the management of AML[218][219]. The use of some other antibodies with conjugates such as CD13, FLT3, and C-type lectin-like molecule 1 (CLL-1) as well as antibodies combined with NK cell transfer have shown promise for the management of AML[220][221][222].

Antibodies targeting NK cell inhibitory receptors

NK cell's inhibitory receptors play an important role in immune cell recognition as well as tumour escape[223]. These inhibitory receptors, including the MHC-I-specific inhibitory receptors (KIRs, LIRs), and immune checkpoints (PD-1, CTLA-4, TIGIT, Siglec-7, TIM-3) are known to cause NK cell dysfunction as earlier discussed. While anti-KIRs have not proved to be efficacious in clinical trials, the use of checkpoint inhibitors which are approved for some solid tumours has shown some efficacy in AML[192][193][224]. In a phase 2 trial of nivolumab and azacitidine in pre-treated AML patients, the ORR was 33% with a CR of 22%[225]. Another phase 2 trial of nivolumab in combination with cyclophosphamide in R/R AML patients has recently been concluded (NCT03417154). As for the anti-TIM-3 antibody sabatolimab (MBG453) in a phase 1 clinical study (with decitabine or azacitidine) it was safe and well tolerated in HR-MDS and AML[226][227]. At present, 3 of 11 clinical studies involving TIM-3 inhibitors in AML/MDS have completed recruitment (NCT03066648, NCT03946670, and NCT04266301).

BiKE and TriKE

Bi-specific killer cell engager (BiKE) and tri-specific killer cell engager (TriKE) are a new class of potential immunotherapeutic agents that are the NK counterparts of bispecific T cell engager (BiTE). They also function as immunological synapses between NK cells and cancer cells just like T cell engager. T cell engagers can activate T cells, and cause proliferation, cytokine release and cancer cell death by bypassing the T cell receptor (TCR) and MHC contact. BiTE has been very efficacious in the management of haematological malignancies; despite this, CRS and Immune effector cell-associated neurotoxicity syndrome (ICANS) are two very serious adverse events that can cause morbidity and mortality in patients treated with BiTE. On the other hand, NK engagers primarily activate NK cells by targeting CD16, NKp46, or NKG2D on NK cells[228][229][230]. BiKEs and TriKEs have been shown to have good activity against several

cancers. Preclinical studies of CD16xCD33 BiKE show that they can facilitate adequate NK cell activation leading to the destruction of AML cell lines and primary AML cells[231][232]. A TriKE which consists of a modified human IL-15 incorporated into the CD16x CD33 BiKE was shown to induce significant NK cell cytotoxicity, degranulation, and cytokine production against CD33+ HL-60 cells[233]. Further studies in second-generation TriKE also showed they were more potent than the first-generation, and induced cell death in a patient-derived xenograft (PDX) AML tumour model as well as both AML cell lines and primary patient-derived AML blasts [234][235]. Reusing et al also showed that paediatric AML and biphenotypic ALL primary cells responded to BiKE treatment[236]. A phase 1/2 clinical trial (NCT03214666) using a designed TriKE was reported to demonstrate significant bone marrow blast level reductions in AML and MDS patients without the need for expensive progenitor-derived or autologous/allogeneic cell therapies[237]. Taken together, these immunotherapies have potential in AML management just like their cousin BiTE.

Cytokines

In the developmental spectrum of NK cells, IL-2, IL-12, IL-15, IL-18 and IL-21 are key players in the proliferation, activation and effector functions of NK cells. IL-2 was the first drug described to enhance NK cell activity, and to date, is the only Food and Drug Administration (FDA) approved cytokine for the treatment of cancer patients[238]. However, IL-15 is another very promising cytokine for the activation of NK cells. It is reported that in the ex vivo stimulation of NK cells in AML patients, 50 ng/mL of IL-15 or 10 ng/mL of IL-2 was optimal for the recovery of its function through the upregulation of activating receptors NKp30, NKp46, NKG2C and NKG2D[238][239]. Though it can expand and activate NK cells, studies have shown that 1L-2 may not have adequate clinical efficacy as a monotherapy in AML patients[240][241]. However, IL-2 in conjunction with some other therapies have shown clinical efficacy in AML, especially as a maintenance therapy[242][243][244][245]. Clinical studies have shown that IL-15 expands NK cells in cancer, however, high expression of IL-15 is associated with CNS disease and neurocognitive impairment in ALL[246][247][248][249][250][251]. IL-15 has also been shown to increase the cytotoxicity of NK cells in patients with AML[252]. In a phase 1 clinical trial, the IL-15 superagonist complex ALT-803 given as a monotherapy to AML patients who relapsed after allogeneic HSCT was observed to be safe and well-tolerated and with one CR (NCT01885897)[253]. A phase 1 trial of ALT-803 in solid tumours was also reported to have produced a significant rise in NK cell numbers (NCT01727076) [254]. However, a recent clinical study by Berrien-Elliott et al reported that systemic IL-15 can promote allogeneic cell rejection in R/R AML patients treated with natural killer cell adoptive therapy(NCT03050216 and NCT01898793)[255]. A CAR-NK cells co-expressing transgenes for the NKG2D CAR and interleukin-15 (IL-15) was recently developed, and it shows enhanced in vitro and in vivo activity in an AML mouse model[256]. With regards to IL-21, a membrane-bound 1L-21 adoptive NK product was shown to reduce AML burden in vivo and had better OS in human subjects with AML[257]. IL-21 was also found to inhibit primary AML stem cells in vitro with the enhancement of cytarabine treatment[258]. Currently, two studies are recruiting for IL-21 trial in AML (NCT04220684) (NCT02809092)[259][260].

NK cells pre-activated with a cocktail of cytokines (IL-12, IL-15 and IL-18) show sustained anti-leukaemia responses to restimulation for weeks to months irrespective of the inhibitory KIR-KIR ligand interactions. These memory-like NK cells have been reported to have antineoplastic potentials. A clinical trial using adoptive transfer of cytokine-induced memory-

like (CIML) NK cells in R/R AML patients was reported to induce remission without serious adverse events[261]. The ongoing donor transfer of CIML NK cells (NCT03068819) in R/R paediatric and young adult AML patients has reported encouraging data of sustained CR[262][263]. Thus, cytokine products are therapeutic candidates for AML management. Some of these clinical trials are listed on the table (Table 1).

Table 1. Some clinical trials of NK cells cellular therapies.

Identifier	Phase	Condition	NK cell source	Intervention	Status	Outcome
NCT02809092	I/II	R/R AML	Haploidentical NK	Before treatment with chemotherapy	Completed	78.6% overall response; 50.0% CR; CNS responses in 4 patients
NCT01385423	I	Refractory AML	Haploidentical NK	Before treatment with lymphodepleting chemotherapy; After treatment with rhIL-15 intravenously (0.3-1.0 mg/kg)	Completed	Robust NK expansion in 36% of patients at day 14; CR in 32% of patients
NCT00703820	II	Paediatric AML	Haploidentical NK	Before treatment with lymphodepleting chemotherapy and rhIL-2 subcutaneously	Completed	None
NCT02763475	II	Paediatric AML	Haploidentical NK	Before treatment with lymphodepleting chemotherapy and rhIL-2 subcutaneously	Completed	CR in 6 of 7 patients
NCT05247957	I	R/R AML	CAR-NK cell	Pretreated		Not provided
NCT05272293	I/II	Paediatric AML	Haploidentical NK	Pretreated	Recruiting	Not provided
NCT05256277	I	R/R AML adults	CIML NK cells	Pretreated	-	Not provided
NCT02727803	II	AML, MDS etc	UCB-derived HSPC-NK cell	Treated with Busulfan, Clofarabine, Cyclophosphamide, Fludarabine Phosphate, Melphalan, Rituximab	Recruiting	Not provided
NCT01823198	I/II	AML, MDS etc	PBMC-derived NK cell	IL-2, Busulfan, Fludarabine	Completed	Not provided
NCT04221971	I	AML adults	PBMC-derived NK cell	Pretreated	Completed	1/3 with MRD negative, low dose group; 3/4 response with 1 case of extramedullary recurrence of AML turned negative, middle dose group.
NCT04310592	I	AML adults	Placental-derived HSPC-NK cell (CYNK-001)	Pretreated	Recruiting	Not provided
NCT04623944	I	AML adults	Car-NK cell (NKX101)	Pretreated	Recruiting	Not provided
NCT04901416	I	AML adults	PBMC-derived NK cell (DVX201)	Pretreated	Recruiting	Not provided
NCT04347616	I/II	AML	UCB-NK cells + 1L-2	Pretreated	Recruiting	Not provided
			CAR-NK cell			

NCT05008575	I	R/R AML	(Anti-CD33)	Pretreated	Recruiting	Not provided
NCT05215015	I	AML	CAR-NK cell (Anti-CD33/ CLL1)	Pretreated	Recruiting	Not provided
NCT04220684	I	AML	Haploidentical NK cell (IL-21 expanded)	Pretreated	Recruiting	Not provided
NCT05333705	I	AML	PBMC/ UCB NK cell	-	Recruiting	Not provided
NCT04836390	I	Paediatric AML	Haploidentical NK cell	-	Enrolling by invitation	Not provided
NCT03821519	I/II	AML, MDS etc	CIML NK cells	Pretreated (with allo-HSCT)	Recruiting	Not provided

Nanoparticles in enhancing NK cell therapy

New avenues are being explored for NK cell-based therapies. One such area is nanotechnology and nanomedicine. Nanotechnology is being used to see its feasibility in NK cell expansion and activation. This can be done through several ways including nanoparticle-assisted immunomodulation to enhance NK cell activity, nanoparticle enhancing homing of NK cells, nanoparticles activating NKG2D receptor etc[264]. Several NK cell-based nano-immunotherapies for cancer are actively being developed, and one is currently in phase 2 trial[265][266]. In a study on NK cells, Sanz-Ortega and her colleagues were able to use magnetic nanoparticles (MNPs) to improve the targeting of adoptively transferred NK cells without altering their function[267]. Selenium-containing nanoparticles were in a study used to enhance NK cell function[268]. Nanoengagers were shown to be more effective in activating NK cells than antibodies. In addition, they could augment both NK-activating agents and chemotherapy to achieve a greater intensity of chemoimmunotherapy[269]. Nanoengagers were also created for T cells against a xenograft AML model which effectively activated T cells and induced AML cell death both in vitro and in vivo[270]. This shows the potential of nano-immunotherapies in the management of haematological malignancies. Very recently, Zeinabad engineered an NK cell mimic nanoparticle which was functionalized against an anti-CD38 antibody (Daratumumab). It showed in vitro activity against AML cell lines, patient-derived AML cells ex vivo and CD38-positive AML cells in vivo, in a disseminated AML xenograft model[271]. This same nanocoupling was also successfully used to target some haematological cancer cell lines. NK cell-based nano-immunotherapy is still in its infancy, but it is believed it can be one of the arsenals against AML shortly.

Conclusion and Future Perspective

NK cell-based therapies have shown potential as viable and strategic therapeutics in the management of AML in the future. The various preclinical and clinical studies on NK cells conducted so far show a challenging but achievable feat. However, getting the different NK cell therapeutic forms to do what they are for against an enemy that is highly

heterogeneous like AML is a priority. Compared to T cell therapies, NK cells have some advantages. NK cell tumour detection is not strictly based on MHC recognition, besides, it can also mediate ADCC. NK cells also provide a better safety profile compared to T cell therapies, including a lower incidence of GvHD, CRS and ICANS. Though NK cells have a limited lifespan, they are easy to prepare under good manufacturing practice standards, which implies an "off the shelf" benefit and a universal administration for the management of patients in a short period. However, NK cell cellular-based therapies are still faced with some challenges which include how to ensure sustained in vivo expansion and proliferation of NK cells as a result of their short lifespan in patients which leads to a short duration of response. How to stop the various immune escape mechanisms used by AML to evade detection and cell death, especially through the creation of an immunosuppressive tumour microenvironment. The efficient transduction of CAR-NK cells will also need to be addressed. I believe some of these challenges can be improved through research, both basic and translational.

While several phase 1/2 clinical trials have been done, a phase 3 trial is yet to be done. This will require a well-designed randomized clinical trial with an adequate sample size to help determine the optimal dosing and therapeutic efficacy of each NK cell therapy in AML. This may also make us know the most efficacious NK cell therapy in AML. We may also need to know the timing of the therapies. Should AML patients be given induction remission or perhaps consolidation to another induction remission therapy? What about maintenance? And how many cycles should be given for each stage?

Given our current knowledge of the various NK cellular therapies, it may be reasonable to try them as a combination therapy which may prove efficient in terms of stimulation of proliferative and effector functions as well as in vivo sustainability to kill AML cells. The CIML NK cells may be useful in this aspect due to their ability to prolong the duration of NK cells in vivo. In addition, a combination of standard AML therapies with NK cellular therapies may be a reasonable option that can provide some form of synergism and enhance NK cells to fight AML. Immunomodulatory drugs such as lenalidomide and thalidomide have been shown to enhance NK cell functions through the release of IL-2 and IFN-γ from surrounding T cells and dendritic cells or using drugs that make AML cells more sensitive to NK cell-mediated lysis, such as bortezomib.

In conclusion, NK cell cellular-based therapies have a bright prospect in the management of AML, and with more clinical research it may soon be a reality.

Statements and Declarations

Ethical Approval and Consent to participate

Not applicable

Availability of data and materials

All data and materials for this work are included in the body of the work.

Disclosure interests

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