

Emerging Cell Therapy Approaches in Relapsed/Refractory Acute Myeloid Leukaemia in Older Adults

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Approximately 30% of patients with acute myeloid leukaemia (AML) experience a lack of response to frontline therapy, as the therapy fails to achieve its intended effect, and those who initially respond have a high relapse rate. Relapsed or refractory AML in older adults tends to harbour high-risk cytogenetics, higher mutational burdens, antecedent haematologic disorders and myeloid dysplastic syndrome features. This is often further complicated by the presence of additional medical comorbidities, especially with increasing age. Drug resistance due to inflammation, alterations in leukaemic stem cells and bone marrow stromal cells tempers the long-term outcomes of chemotherapeutic and targeted therapies. Cell therapy approaches have emerged as a possible solution, including adaptations of conditioning chemotherapy to haematopoietic stem cell transplantation to increase tolerability and efficacy. Here, we review the considerations for the need and application of these novel therapies.

Keywords

Cell therapy, cytogenetics, drug resistance, immunotherapy, leukaemia, stem cell transplantation

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Acute myeloid leukaemia (AML) is a heterogeneous haematologic cancer associated with clonal expansion of myeloid blasts in the bloodstream, bone marrow and other tissues. AML is associated with blasts expressing various complex molecular and cytogenetic alterations, which play an important role in disease prognostication.

The median age of AML diagnosis is 67 years with nearly 30% of patients above the age of 75 years.¹ The National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program has estimated 20,050 new cases and 11,540 deaths in 2022, and the disease is most frequently diagnosed among people 65–74 years of age.^{2,3} While the 5-year survival has increased since the 1970s, the most recently reported survival rate for AML (2012–2018) was 30.5%, compared with 70.8% for acute lymphocytic leukaemia, which includes both adult and paediatric cases.

Patients diagnosed with AML are risk stratified for survival outcomes on numerous factors, including age and performance status, prior history of myelodysplasia or myeloproliferative disorders, prior exposure to cytotoxic agents or radiotherapy and, importantly, cytogenetic and molecular risk factors.^{4,5} Cytogenetic and molecular mutations are detectable in approximately 50–60% of newly diagnosed AML cases and confer survival outcomes that are categorized into favourable, intermediate and poor-risk groups according to the European LeukemiaNet criteria.^{5,6} These criteria were determined based on outcomes from those receiving intensive therapy, mostly in individuals below 60 years of age. The mutational spectrum of older patients with AML (>60 years) varies drastically compared with individuals below 60 years. The incidence of *TET2*, *DNMT3A*, *SRSF2*, *ASXL1*, *TP53* and *SF3B1* is higher, indicating that these genes were associated with age-related haematopoiesis.⁷ A higher incidence of isocitrate dehydrogenase (IDH) mutations is also reported in older patients, which is a strong genetic predictor for shorter survival.⁸ However, with the advent of IDH inhibitors, the adverse impact of this mutation has become less clear. Thus, older patients are more likely to have more comorbidities, worse performance status, higher likelihood of preceding myeloid dysplastic syndrome (MDS) features and high-risk cytogenetic and molecular risk features, and have lower response rates as seen in response to intensive induction chemotherapy 40–60% in patients >60 years versus 60–85% in patients ≤60 years.^{9,10} Although patients with intermediate and high-risk cytogenetics are referred for consolidation with allogeneic haematopoietic stem cell transplantation (HSCT), older and more frail patients are often deemed ineligible due to risk of transplant-related mortality or higher risk of relapse with reductions in conditioning intensity. Thus, novel immunotherapies and approaches to cell therapy have emerged to address this unmet need.

The current approach to treatment for newly diagnosed and relapsed/refractory AML (R/R AML) is patient-dependent – based on patient values, preference and tolerability of therapy. For the fit

patient, the goal of treatment is curative, inducing complete remission (CR) and reducing the risk of relapses with high-intensity chemotherapy followed by consolidation therapy. Nearly 50% of patients with AML who have attained CR eventually relapse and 40–60% of them are individuals above 60 years of age.¹¹ This is attributed to metabolic adaptation causing drug resistance and tolerance and is being actively studied.

Drug resistance and bone marrow microenvironment in older patients with acute myeloid leukaemia

The bone marrow microenvironment (BMME) is a complex system consisting of a heterogeneous group of haematopoietic and non-haematopoietic cells. These cells play an important role in cell renewal and differentiation, thereby initiating and propagating leukaemic cells.^{12,13} They also play an important role in drug resistance, which is a potential concern with targeted agents.¹⁴

The bone marrow stroma in older patients, having been modified due to pre-existing chronic medical conditions and medications, can release various chemokines that promote the survival of leukaemic clones and contribute to treatment resistance.¹⁵

Inflammation

Inflammation is a physiological process often associated with elderly patients, as many have chronic inflammatory diseases, leading to alterations in the intracellular signals and the release of cytokines such as interleukin (IL)-1 and IL-6, which cause remodelling of the BMME, promoting drug resistance and refractory leukaemogenesis.¹⁶ Various targeted agents, such as FLT3 inhibitors combined with steroids, have been effective in downregulating inflammatory pathways, thereby preventing the development of drug-resistant mutant clones.¹⁷ Similarly, the NF- κ B inflammatory pathway has been postulated to be the culprit for venetoclax/azacitidine resistance.¹⁸

Intrinsic drug resistance

The role of intrinsic drug resistance in older individuals is poorly understood and needs further investigation. Possible hypotheses include increased expression of adenosine triphosphate-binding cassette, resulting in direct efflux of the drugs, and genetic mutations associated with target binding sites.¹⁹ The emergence of multiple mutations in the binding sites of IDH1 inhibitors, particularly around the crystal substructure, can interfere with the direct action of ivosidenib, reducing its effectiveness as an anticancer agent. This serves as a notable example of this phenomenon.²⁰

Alteration in leukaemic stem cells

Leukaemic stem cells remain in the BMME and are normally in a quiescent state. However, pro-inflammatory markers, such as cytokines, reverse this state and promote proliferation and are leading to relapse in older patients.²¹ Various targets involving mitochondrial metabolism and oxidative phosphorylation have shown promising results in destroying resistant leukaemic stem cells.²² Recent advancements in AML treatment have led to the FDA approval of novel therapies targeting menin, a crucial oncogenic cofactor in leukaemogenesis. Menin inhibitors, such as revumenib (SNDX-5613), showed promise in overcoming leukaemic cell resistance by disrupting menin's interaction with KMT2A fusion proteins (A Study of Revumenib in R/R Leukemias Including Those with an MLL/KMT2A Gene Rearrangement or NPM1 Mutation; ClinicalTrials.gov identifier: NCT04065399).²³ Additionally, these inhibitors have demonstrated efficacy in treating NPM1-mutated AML by targeting the aberrant transcriptional programmes driven by the interaction between menin and mutant NPM1 proteins.^{24,25}

Resistance in bone marrow stromal cells

Bone marrow stromal cells contribute to resistance by creating a protective microenvironment that shields leukaemic cells from T-cell-mediated lysis, thereby supporting immune evasion. This mechanism has been widely studied in FLT3 inhibitors conferring resistance by altering the fibroblast growth factor and signal transducer and activator of transcription pathways.²⁶ Direct contact between stromal cells and leukaemic cells confers resistance by influencing intracellular processes within the leukaemic cells, promoting survival and resistance. This is possible by the over-expression of adhesion molecules like vascular cell adhesion molecule-1, which promotes increased survival through rapid proliferation.^{27,28}

While drug resistance continues to remain a challenge, the role of immunotherapeutic agents has been widely explored in the management of patients with AML. Novel immune therapy agents were introduced to identify newer mechanisms of action that have a lower threat of developing drug resistance.

Immunotherapies for acute myeloid leukaemia Targeted therapy using T-cell engagement

Emerging immunotherapeutic approaches using the current concept include bispecific T-cell engagers (BiTEs) and dual affinity retargeting proteins (DARTs). BiTE antibodies are developed based on monoclonal antibodies targeting two immune-related molecules, such as CD3 and one tumour antigen, simultaneously. Bispecific antibodies are composed of two independent polypeptides, variable light chain and variable heavy chain, both of which are components of the fragment variable (Fv), which are tethered together and interact preferentially, as mentioned above.²⁹ DART molecules redirect the leukocytes to eliminate tumour cells. They are solely dependent on a stable single-chain Fv produced by protein engineering, which eliminates the need for independent polypeptides as in BiTE therapy, thereby providing better stability and potency.³⁰

Two products, AMG 330 (CD3 $\times$ CD33) and flotetuzumab, are immune therapies that were previously evaluated for the treatment of R/R AML. However, both therapies are no longer in clinical trials currently being evaluated for the treatment of R/R AML. In a phase I trial with AMG 330, a complete response (CR) rate of 17% was observed, with more than half of the patients expressing poor cytogenetics.³¹ Flotetuzumab is a DART. They act by binding to CD123, an IL-3 receptor that is present in leukaemic cells.³² A study using flotetuzumab in relapsed AML has shown a CR rate of 26.7% with a median survival of 10.2 months. The most common adverse effect was cytokine release syndrome (CRS) treated with dexamethasone and tocilizumab without any reported fatalities.³³

While AMG 330 and flotetuzumab are no longer in trials, ongoing studies are investigating other promising therapies, including vibecotamab (XmAb14045) for MRD-positive AML and MDS after hypomethylating agent failure (A Study of Vibecotamab [XmAb14045] in Subjects with MRD-positive AML and MDS Following Hypomethylating Agent Failure; ClinicalTrials.gov identifier: NCT05285813), CD19 chimeric antigen receptor (CAR)-T cells in R/R AML expressing CD19 (A Study of CD19 Chimeric Antigen Receptor [CAR] T Cells in Relapsed/Refractory AML Expressing CD19; ClinicalTrials.gov identifier: NCT06649227), CLN-049 in R/R AML or MDS (A Study of CLN-049 in Relapsed/Refractory AML or MDS; ClinicalTrials.gov identifier: NCT05143996) and AFM28 in relapsed/refractory AML (A Study of AFM28 in Relapsed/Refractory AML; ClinicalTrials.gov identifier: NCT05817058).^{34–37}

The data on the toxicity profile associated with T-cell engager therapies, which include BiTE and DART, have been mainly obtained through clinical trials. From the available data, patients studied received multiple previous treatment lines. Data were not stratified by age.^{31,33,38} More frequently reported adverse events were grade 1–2 fever, neutropenia, nausea and elevated liver enzymes.^{33,39}

CRS, commonly observed with bispecific antibodies and CAR-T-cell infusions, is due to T-cell activation, producing cytokines including IL-6 and interferon-gamma.⁴⁰ The clinical presentation can range in severity. Mild CRS may manifest as fever, diarrhoea or shortness of breath. Severe CRS may resemble macrophage activation syndrome and render the patient critically ill.⁴¹ Various treatment algorithms have been developed, which include the use of prophylactic dexamethasone or treatment with tocilizumab, an anti-IL-6 antibody.^{40,42} The reported incidence for AMG330 in phase I and II trials is 70%, and nearly all patients with flotetuzumab developed infusion-related cytokine reactions (grades 1 and 2).^{31,33}

Neurotoxicity, or immune effector cell-associated neurological syndrome (ICANS), is also a common adverse effect of BiTE and CAR-T-cell therapy, with around 52% of patients receiving blinatumomab experiencing mild symptoms in the form of tremors.³⁹ However, one should be vigilant for severe symptoms, including seizures, aphasia and encephalopathy.⁴³ Severe CRS and neurotoxicity can lead to CRS-related deaths or treatment-related mortality. Patients can die from neurotoxicity-related cerebral oedema or CRS-related causes.^{44,45} Furthermore, no change in the efficacy of these agents was determined due to these toxicities.

Although our data on the use of T-cell engager therapies are still from early-phase clinical trials, the biggest challenges encountered are patient selection and the optimal timing of using these agents, such as for MRD eradication versus relapse or failure. Performance status and associated concomitant medical conditions, such as heart failure, severe dementia and end-stage renal disease, remain an important factor during patient selection, especially taking into consideration CRS and ICANS, which are brought about by severe cytokine release and can alter the volume status in older patients. T-cell engagers do not require lymphodepleting agents prior to their use, which is a great advantage with these agents. Since this is an evolving field, updated data from clinical trials are required to form a consensus on specific nuances associated with its use. We have data for diseases other than AML treated with CAR-T-cell therapy that describe increased risk for older patients. Extrapolating results from these data continue to show benefits in terms of survival.^{46–48} Therefore, it is extremely essential to consider geriatric assessment prior to patient selection.⁴⁹

Chimeric antigen receptor T-cell therapy

The CAR construct is a single-chain variable fragment (scFv) anchored to a T-cell transmembrane domain (e.g. 4-1BB and CD28) and intracellular signalling domain. The scFv is derived from a monoclonal antibody targeted to the cancer cell. Targets for AML include CD33, CD123, CD13, CD123, FLT3, NPM1, KIT, BCL-2, IDH1, IDH2, CD34, TP53, VEGFR and CXCR4. Once expressed in T cells, CAR-modified T cells acquire properties that exert immediate and long-term effects.⁵⁰

CAR-T-cell therapy has shown great promise for haematological malignancies, but its application in AML presents unique challenges, including antigen heterogeneity and the risk of toxicity to normal

haematopoiesis.^{51,52} Differentiating between autologous and allogeneic CAR-T-cell therapies highlights innovations, such as universal CAR-T cells, which offer advantages, such as broader availability, reduced manufacturing times and cost-effectiveness.⁵² Efforts to mitigate off-target effects have led to advanced engineering approaches, including the use of CRISPR-Cas9 technology to enhance safety and efficacy.⁵³ For example, base-edited CD7 CAR-T cells developed at the UCL Great Ormond Street Institute have demonstrated promising results and are now being evaluated in a clinical trial (Base-Edited CAR7 T Cells to Treat T Cell Malignancies; ClinicalTrials.gov identifier: NCT05397184).^{54,55} Similarly, the Shenzhen Geno-Immune Medical Institute is conducting trials of third-party CAR-T cells targeting multiple antigens, such as CLL-1, CD33, CD38 and CD123, showcasing strategies to address antigen escape.^{56,57}

Various studies (*Table 1*) have contributed to the understanding and optimization of CAR-T-cell therapy for AML.^{31,33,34,43–46,57–99} Research on cooperative CARs has shown they can effectively target AML cells while minimizing escape and sparing normal haematopoietic stem cells.¹⁰⁰ Investigations into cytokine-mediated resistance in AML have identified mechanisms of therapy failure and proposed solutions to enhance treatment efficacy. CD70-specific CAR-T cells have demonstrated potent activity against AML without affecting normal haematopoiesis, highlighting a promising target for safer therapies.¹⁰¹ Genetically modified allogeneic CD7-targeting CAR-T cells have shown enhanced efficacy in clinical trials for relapsed or refractory cases.¹⁰² Adapter CAR-T cells have been developed to counteract T-cell exhaustion and improve targeting flexibility, while dual-receptor platforms combining antibody-based and costimulatory mechanisms have achieved significant specificity and potency.¹⁰³ Additionally, the use of CRISPR combined with transposon-based technologies has optimized universal allogeneic CAR-T cells for AML treatment.¹⁰⁴ These advances collectively underscore the potential of CAR-T-cell therapy for AML, driven by innovative engineering strategies and targeted approaches to address its unique challenges.

Tumour-selected antibodies and antigen retrieval system is being developed to isolate nanobodies that bind to CD19 and TIM3-expressing AML cells. CD33- and CD123-directed CAR-T products are being investigated in phase I/II trials (CLL1-CD33 cCAR in Patients with Relapsed and/or Refractory, High Risk Hematologic Malignancies; ClinicalTrials.gov identifier: NCT03795779; and Study Evaluating Safety and Efficacy of UCART123 in Patients with Relapsed/Refractory Acute Myeloid Leukemia; ClinicalTrials.gov identifier: NCT 03190278).^{60,61} Similarly, a phase I trial using point-of-care manufactured CD19/20/22 CAR-T cells (Genetically Engineered Cells [Anti-CD19/CD20/CD22 CAR T-cells] for the Treatment of Relapsed or Refractory Lymphoid Malignancies; ClinicalTrials.gov identifier: NCT05418088) is being evaluated for relapsed and refractory acute leukaemias.^{62,105} CD33-directed treatment was recently used in a patient, which showed a significant lowering of the blast burden from 50 to 6% in weeks. Unfortunately, the patient died of disease progression and did experience CRS.¹⁰⁶ A phase I study evaluating NKG2D-based CAR-T (Study in Relapsed/Refractory Acute Myeloid Leukemia or Myelodysplastic Syndrome Patients to Determine the Recommended Dose of CYAD-02; ClinicalTrials.gov identifier: NCT04167696) exhibited adequate safety with no grade 3-related toxicities noted because of the agent.^{97,98} Adverse events described with this therapy includes neutropenia, hepatotoxicity.⁹⁸

Table 1: Summary of recent and ongoing trials using cellular therapies in AML^{31,33,34,57–99}

Drug/cell therapy	Mechanism	Patient population	Phase	Results	NCT
Flotetuzumab	Anti-CD3×CD123 (DART)	92 patients with R/R AML	I and II	27% CR; median OS 10.2 months among responders; 100% CRS	NCT02152956 ³³
AMG 330	Anti-CD3×CD33 (BiTE)	55 patients with R/R AML	I	19% ORR; 60% CRS	NCT02520427 ³¹
Vibecotamab	Anti-CD3×CD123 (BiTE)	104 patients with R/R AML	I	14% ORR (4% CR); 59% CRS	NCT02730312 ³⁴
AMG 673	Anti-CD3×CD33 (BiTE)	30 patients with R/R AML	I	44% with blast reduction; 50% CRS	NCT03224819 ⁵⁸
Apamistamab	Iodine-131 radiolabelled conditioning for HSCT	50 patients with R/R AML	III	75% CR at day 30 post-transplant versus 17% in conventional arm; less grade 3 toxicity	NCT02665065 ⁵⁹
CLL1-CD33 CAR-T	CAR-T	R/R AML	I and II	Ongoing	NCT03795779 ⁶⁰
UCART123	CAR-T	R/R AML	I	Ongoing	NCT03190278 ⁶¹
CD19/20/22 CAR-T	CAR-T	Acute leukaemia/lymphoma	I	Ongoing	NCT05418088 ⁶²
JNJ-67571244	CD3×CD33 (BiTE)	R/R AML	I	Ongoing	NCT03915379 ⁶³
APVO436	CD3×CD123 (BiTE)	R/R AML	I	Ongoing	NCT03647800 ⁶⁴
MCLA-117	CD3×CLEC12A (BiTE)	R/R AML	I	Ongoing	NCT03038230 ⁶⁵
AMG 427	CD3×CD135 (BiTE)	R/R AML	I	Ongoing	NCT03541369 ⁶⁶
GTB-3550	CD16/IL-15/CD33 (TriKE)	R/R AML	I and II	Ongoing	NCT03214666 ⁶⁷
JSP191	Actinium-labelled CD45 antibody + anti-CD117	AML/MDS	I	Ongoing	NCT04429191 ^{68,69}
Anti-ILT3 CAR-T	CAR-T	R/R AML (M4/M5)	I and II	Ongoing	NCT04803929 ⁷⁰
Anti-Siglec-6 CAR-T	CAR-T	R/R AML	I and II	Ongoing	NCT05488132 ⁷¹
Anti-Tim-3/CD123 CAR-T	CAR-T	R/R AML	I	Ongoing	NCT06125652 ⁷²
CAR-T CD19	CAR-T	AML with t(8;21) and CD19 ⁺	I	Ongoing	NCT04257175 ⁷³
Anti-FLT3 CAR-T	CAR-T	R/R AML	I	Ongoing	NCT06760260 ⁷⁴
Sequential CD33/CD123	CAR-T	R/R AML	I	Ongoing	NCT06420063 ⁷⁵
Universal CAR-T	CAR-T	R/R AML	I and II	Ongoing	NCT05995041 ⁵⁷
FLT3 CAR-T	CAR-T	R/R AML	I	Ongoing	NCT06786533 ⁷⁶
EX02 CAR-T	CAR-T	R/R AML	I	Ongoing	NCT06642025 ⁷⁷
CT0991	CAR-T	R/R AML	I	Ongoing	NCT06709131 ⁷⁸
Optimized CD33 (FL-33)	CAR-T	R/R AML	I	Ongoing	NCT06326021 ⁷⁹
CD7 CAR-T	CAR-T	R/R CD7 ⁺ haematologic malignancies	I	Ongoing	NCT05454241 ⁸⁰
CLL1/CD38 dual CAR-T	CAR-T	R/R AML	I and II	Ongoing	NCT06110208 ⁸¹
Base-edited CAR-T	Base-edited CAR-T	AML ahead of Allo-HSCT	I	Ongoing	NCT05942599 ⁸²
CD70 CAR-T	CAR-T	CD70 ⁺ haematologic diseases	I	Ongoing	NCT04662294 ⁸³
CD33KO-HSPC	Gene-editing + CAR-T	R/R AML	I	Ongoing	NCT05945849 ⁸⁴
CATCHAML	CD123-directed CAR-T	R/R AML	I	Ongoing	NCT04318678 ⁸⁵
BG1805	CAR-T	R/R AML	I	Ongoing	NCT06118788 ⁸⁶
TAA05	FLT3-targeted CAR-T therapy	R/R AML	I	Ongoing	NCT05017883 ⁸⁷
WU-CART-007	Anti-CD7 allogeneic CAR-T cells	CD7 ⁺ haematologic malignancies	I	Ongoing	NCT05377827 ⁸⁸
CD371 CAR-T	CD371-targeted CAR-T cells	R/R AML	I	Ongoing	NCT06017258 ⁸⁹
VCAR33	Donor-derived anti-CD33 CAR-T cells	R/R post-allogeneic HCT AML	I	Ongoing	NCT05984199 ⁹⁰
CAR-T + dendritic cells	Combination CAR-T and dendritic cells	R/R AML	I	Ongoing	NCT03291444 ⁹¹
ADCLEC.syn1	ADCLEC.syn1 targeted therapy	R/R AML	I	Ongoing	NCT05748197 ⁹²
Universal 4SCAR7U	CAR-T	CD7 ⁺ malignancies	I and II	Ongoing	NCT05995028 ⁹³
PLAT-08	SC-DARIC33 CAR-T	R/R AML	I	Ongoing	NCT05105152 ⁹⁴
CRISPR-edited CAR-T	CRISPR-edited CLL-1-directed CAR-T	R/R AML	I	Ongoing	NCT06128044 ⁹⁵
Anti-CD7 allogeneic CAR	Allogeneic CAR-T	CD7 ⁺ haematologic malignancies	I	Ongoing	NCT05377827 ⁹⁶
CYAD-02	Allogeneic CAR-T	R/R AML/MDS	I	Ongoing	NCT04167696 ^{97,98}
Allo-RevCAR01-T	Allogeneic CAR-T with CD123 target module	CD123 ⁺ haematologic malignancies	I	Ongoing	NCT05949125 ⁹⁹

AML = acute myeloid leukaemia; BiTE = bispecific T-cell engager; CR = complete response; CRS = cytokine release syndrome; DART = dual affinity retargeting protein; HCT = haematopoietic transplant; HSCT = haematopoietic stem cell transplant; MDS = myeloid dysplastic syndrome; NCT = National Clinical Trial; ORR = overall response rate; R/R AML = relapsed and refractory acute myeloid leukaemia.

Natural killer cell-based therapy: Bispecific and trispecific engagers in acute myeloid leukaemia

The use of natural killer cell (NK)-based therapies in leukaemias has been accelerating and expanding over the last few years. NK cells belong to the innate immune system and exert their cytotoxic effects by surface inhibitory receptors, such as natural killer group 2A and natural cytotoxic

receptors.^{107,108} In AML, leukaemic cells escape NK cell-mediated identification and destruction; hence, modifying dysfunctional NK cells by tapping into the tumour microenvironment is the basis of adoptive NK cell therapies in AML.^{109,110} The current strategies for using NK cells in AML include adoptive NK cell transfer in the haematopoietic transplant (HCT) and non-HCT setting, CAR-NK cells and the newest forms of therapy

called bispecific (BiKE) and trispecific engagers (TriKE). In the HCT setting, transplants from NK alloreactive donors have shown anti-leukaemic effects through graft-versus-leukaemic effect and protection against graft-versus-host disease (GVHD) through depletion of antigen-presenting cells and IL-10 production.^{111,112} Various phase I studies are underway investigating CD3-depleted NK cell infusions, which have demonstrated reduced leukaemic progression when compared with patients without NK cell infusion.^{113,114} NK cell infusions have been studied in the form of haploidentical NK cell transfer with high-dose chemotherapy in R/R AML when transplant is not an option.¹¹⁵ Newer phase I studies are underway using placental-derived NK cells and clonal-engineered products (FT516 in Subjects with Advanced Hematologic Malignancies; ClinicalTrials.gov identifier: NCT04023071; and Phase I Study of PNK-007, an Allogeneic, Off-the-shelf NK Cell in Relapsed/Refractory Acute Myeloid Leukemia; ClinicalTrials.gov identifier: NCT02781467).^{116,117} CAR-modified NK cells called CAR-NK products are being considered due to favourable toxicity profiles and low manufacturing costs compared with T cells.¹¹⁸

Bispecific and trispecific engagers are recombinant agents that form synapses between NK and tumour cells. Phase I/II trials using CD16×CD33 BiTE and CD16×NKG2D BiTE are underway for patients with AML.^{119,120} Similarly, CD16×CD33×CD33 TriKE is being evaluated for the treatment of CD33-positive R/R AML (GTB-3550 [CD16/IL-15/CD33] Tri-specific Killer Engager [TriKE] for the Treatment of High-risk Myelodysplastic Syndromes, Refractory/Relapsed Acute Myeloid Leukemia, and Advanced Systemic Mastocytosis; ClinicalTrials.gov identifier: NCT03214666).¹²¹ Current BiKEs and TriKEs display great potential, but efforts need to be made to improve their efficacy. This can be achieved by improving access to trials and by appropriate selection of patients.

Modern approaches in haematopoietic cell transplantation for acute myeloid leukaemia

Allogeneic stem cell transplant remains the standard of care for patients with intermediate and adverse risk disease after the first CR.¹²² Although the myeloablative condition has evidence of improving overall survival (OS) in patients with AML and MDS, it does come with a risk of serious toxicities that can cause mortality in geriatric patients.¹²³ A great unmet need in the field of transplant is its use in R/R AML. Techniques using 'sequential' conditioning with short-course induction chemotherapy followed by reduced intensity conditioning have shown promise. These regimens aim to gain disease control while limiting the conditioning toxicity in older patients. Several of these approaches have shown improvements in tolerability, such as with fludarabine, cytarabine and amsacrine or treosulfan/fludarabine, but the high risk of disease relapses remains.^{124,125} The largest phase III trial using treosulfan as of yet did show improved 2-year survival outcomes with lower toxicities but higher relapse rates and GVHD.^{125,126} To further mitigate relapse, ongoing clinical trials are studying the addition of targeted agents, including venetoclax (BCL-2 inhibitor) and Sorafenib (FLT-3 inhibitor), to conditioning in older relapsed patients with AML (Study of Venetoclax in Combination with Conditioning Regimen for Patients with Acute Myeloid Leukemia or Myelodysplastic Syndrome Undergoing Stem Cell Transplantation; ClinicalTrials.gov identifier: NCT02250937; and Study of Sorafenib with Busulfan and Fludarabine in Patients with Relapsed or Refractory Acute Myeloid Leukemia Undergoing Stem Cell Transplantation; ClinicalTrials.gov identifier: NCT03247088).^{127,128} However, the ASAP (Acute Myeloid Leukaemia Study of Salvage Chemotherapy Versus Allogeneic Stem Cell Transplantation; ClinicalTrials.gov identifier: NCT02461537) trial highlights the benefits of employing HSCT early in the treatment course of R/R AML without sequential conditioning.^{129,130} It was found that OS at 1 year and 3 years was comparable in patients receiving upfront remission induction

conditioning chemotherapy and HSCT versus disease control treatment prior to sequential conditioning, with survival rates of 71.9 versus 69.1% at 1 year and 54.2 versus 51% at 3 years, respectively.¹²⁹

Moreso, for even the most frail or highest risk disease patients, HSCT is becoming more accessible using targeted radioimmunotherapy (RIT) conditioning. Radionucleotides are effective because acute leukaemic cells are sensitive to ionizing radiation.¹³¹ The advantage of radionucleotides over traditional radiation is direct radiation towards leukaemic cells, thereby causing limited cytotoxic side effects and improving tolerability, which is essential in individuals above 65 years.¹³² Various beta-emitters like iodine-131, yttrium-90 and rhenium-188 are being studied as potential conditioning agents for HSCT in patients with AML. These particles deliver radiation over a longer distance through their bystander effect, making these agents ideal for conditioning.^{133,134} A series of clinical trials have shown that beta-emitters combined with CD33, CD45 and CD66 antibodies can be used as non-myeloablative conditioning regimens for HSCT in advanced AML.¹³⁵ In an initial randomized phase III trial, patients 55 years and older with active R/R disease were randomized to I-131 conjugated anti-CD45, apamistamab, versus conventional care.⁵⁹ Only 17% of patients on the conventional care attained CR and proceeded to HCT versus 90% on the apamistamab arm, and 75% of patients receiving apamistamab were in CR approximately 30 days after HSCT. This compared highly favourably with the control arm where only 6% of these patients were in remission after standard HSCT. Twenty-two per cent of the patients receiving apamistamab maintained durable CR (dCR) lasting 6 months or more, while none in the control arm achieved a dCR. This demonstrates the difficulty of maintaining durable remissions in this patient population despite being in a CR pre-transplant as in the control arm. The incidence of grade >3 toxicity was also lower (5% versus 30%), with 100% engraftment rates.⁵⁹ The study met its endpoint with 60% of apamastimab patients alive without relapse at 2 years versus 0% of patients on the conventional care arm with dCR >6 months. The secondary endpoints for OS and event-free survival were also met. Other similar studies include using alpha-emitters such as bismuth-213-labelled lintuzumab and actinium-labelled lintuzumab (CD45) and a novel anti-CD 117 antibody, JSP191, in combination with total body radiation and fludarabine. Phase I results preliminarily reported neutrophil engraftment between 19 and 26 days with only grade 2 toxicities. High rates of minimal residual disease (MRD) clearance were observed in 15 out of 17 patients. The final results of the study are pending (JSP191 Antibody Conditioning Regimen in MDS/AML Subjects Undergoing Allogeneic Hematopoietic Stem Cell Transplantation; ClinicalTrials.gov identifier: NCT04429191).^{68,69,136}

An established practice at many transplant centres include use of donor lymphocyte infusions (DLIs) in relapsed AML post-allogeneic transplant. DLIs are lymphocyte concentrates collected from the original stem cell donor; however, the efficacy of these products has been limited and is considered predominantly in first-relapse patients after transplant.¹³⁷ Retrospective data exist showing inferior results of DLIs versus cytoreductive treatment with a 2-year survival of only 9%. Randomized controlled trials are needed to explore this option on a larger scale.¹³⁷

Conclusion

Treatment of R/R AML has evolved with the advent of targeted therapies and newer immunotherapeutic drugs in older patients. However, the cure rate of AML is still unsatisfactory, which has been attributed to evolving drug resistance through various mechanisms and identification of newer complex molecular mutations. The use of targeted molecules

has resulted in better tolerance and response rates but not towards cure without the use of HSCT. Novel cellular therapy approaches, including T-cell engagement and CAR-T-cell therapies, may open the door while increasing the tolerability of HSCT through novel approaches, such as RIT conditioning, which have proven tolerable in highly frail patients with active disease and dCR. More than ever, older patients with R/R AML

have pathways towards prolonged survival. These advances continue to lead to future directions incorporating the use of RIT earlier in the treatment course, expanding access to HSCT for better outcomes, and innovating approaches to enhance the efficacy and tolerance of other immune cell therapies. □

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