Current developments and hurdles in CAR-T cell therapy for acute myeloid leukemia

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SUMMARY

Despite huge progress in the past decades, the overall survival (OS) of patients with acute myeloid leukaemia (AML) remains poor. The treatment options run low for those refractory or intolerant to first and second line treatment or in case of relapse. The need for alternative treatment is great and imperative to further improve the OS of these patients. The success of CAR-T19 therapy for the treatment of B cell acute lymphoblastic leukaemia has demonstrated the feasibility of delivering these therapies, and success in further improving survival rates. Among others, the fundamental biological factor limiting the applicability of CAR-T immuno-therapy in the treatment of AML includes the lack of a leukaemia-specific antigen, or an antigen shared by leukaemia blasts and haematopoietic stem and progenitor cells whose sustained depletion could be clinically tolerated. In this review, we describe the most recent developments, clinical results and challenges in CAR-T cell therapy for AML.

KEY WORDS

CAR-T cell therapy, chimeric antigen receptor, acute myeloid leukaemia, immunotherapy

INTRODUCTION

In the past three to four decades huge progress has been made in the treatment of acute myeloid leukaemia (AML) resulting in steadily improving overall survival (OS) rates. Nevertheless, the 5-year OS in Belgium is 74% in paediatric AML patients and only +/-25% in adult AML patients.[1-3] Allogenic hematopoietic stem cell transplantation (allo-HSCT) is in many cases the only potentially curative treatment option with known side-effects and the risk for graft-versus-host disease (GvHD). Nonetheless, relapse after allo-HSCT is frequent and leaves very few therapeutic options. In addition to GvHD, a graft-versus-leukaemia (GvL) phenomenon has been reported, and has been shown to play a crucial role in further eliminating leukemic cells.[4, 5] Importantly, this GvL effect strongly suggests that AML and other types of leukaemia are susceptible and responsive to the anti-tumour activity of allogenic T cells.[4] This observation has, amongst others, fuelled the development of chimeric antigen receptors (CAR)T cells, which recently have been shown to significantly improve OS rates in case of refractory or relapsed B cell malignancies.[6] CARs are genetically engineered molecules that can both identify specific target proteins on the cell surface of cancer cells as well as trigger multiple T cell functions (i.e. activation, proliferation and memory). It is a cellular therapy, aimed to redirect a patient's or donor's T cells to recognize and destroy tumour cells in a way that is major histocompatibility complex (MHC)-independent. Consequent benefits are high specificity, active trafficking to tumour sites, and durable persistence and expansion in vivo.[7, 8] Nevertheless, direct translation of this technology to AML treatment has been limited. Within this review, we discuss the most recent developments and clinical trial results and highlight the hurdles which should be overcome in order to successfully introduce CAR-T therapy in the treatment of AML.

CAR-T CELL THERAPY

CLINICAL TRIALS AND RESULTS

The first clinical trial of CAR-T cell therapy in AML patients was a phase I study with anti-LeY CAR-T cell therapy in patients with high risk AML, demonstrating a durable persistence in vivo and a good feasibility and safety. Even though the observed efficacy was only limited, the results were very promising and showed the sensibility of AML cells towards CAR-T cell therapy.[9] Meanwhile, various other targets have been identified and used for the manufacturing of specific CAR-T cells. In 2015, a 47-year-old male with relapsed and refractory AML-M2 was treated with CD123-CAR-T cells after conditioning chemotherapy. Examination of the bone marrow on day 20 showed a decrease in blasts from 59% to 45%, and no unexpected toxicities were observed (NCT02159495).[10] These results have led to several still ongoing trials, evaluating the clinical effect of CD123-CAR-T cells (NCT04318678, NCT03766126, NCT03190278, Table 1).

An update of the first-in-human CLL1-CD33 compound CAR (cCAR)-T cell therapy (Figure 1) showed the achievement of complete remission in patients with refractory AML. On disease re-evaluation within 4 weeks post CAR-T cell infusion, 7 of 9 patients were minimal residual disease (MRD) negative by flow cytometry, 2 of 9 had no response, one of which was CD33+/CLL1-, indicating the importance of CLL1 targeting in the CAR-T treatment. Interestingly, multiple patients proceeded to HSCT with a less intense conditioning. In this way, cCAR could be interesting as potential conditioning therapy, when patients are intolerant to total body radiation or high dose chemotherapies.[11] Clinical studies with dual CD123-CLL-1 CAR-T cells are also ongoing (NCT03631576). Single CAR-T cells against CD33 and CLL-1 are currently being investigated in early clinical trials, but no results have been published yet (NCT03971799, NCT01864902, NCT03126864; NCT04219163; Table 1).

Besides CD33 and CD123, other hematopoietic stem cell markers such as CD34, CD38 and CD133 are being investigated as targets in CAR-T development (NCT03473457, NCT04351022). Nevertheless, similar limitations as observed in CD123 and CD33 CAR-T therapy, are to be expected. Furthermore, the use of multi-CAR-T cells with various myeloid markers, such as CD33, CD38, CD56, CD123, Muc1 and CLL-1, is under investigation to optimize efficacy and widen the clinical application (NCT0322264). Current phase I and II clinical trials evaluating CD7-directed CAR-T cells in AML patients are ongoing (NCT04033302), as well as in T cells malignancies (NCT04480788). Results of these trials have not yet been published.

CYAD-01, a CAR-T product based on the receptor NKG2D, was tested in a phase I trial, among which 8 AML patients, and proven to be feasible and safe without preconditioning therapy. There was promising anti-leukemic activity with 42% objective response rate in relapsed and/or refractory AML with 5 out of 7 evaluable patients having clinical benefit. However, clinical benefit seemed to be short-lived and the percentages of blast-reduction were rather low. There was one durable complete molecular remission for more than one year, after bridging to allo-HSCT on day +97 post CYAD-01 injection (NCT02203825).[12] In another phase I trial published in 2019, 12 patients -of which 7 AML patients- were treated with autologous NKG2D CAR-T cells, showing an acceptable safety profile without long-term toxicities, but no clinical efficacy (NCT03018405).[13] Because of the lack of clinical efficacy, the company announced a stop of these clinical trials. Consequently, CYAD-02 was manufactured, a next-generation product in which short hairpin RNA (shRNA) technology is used to silence MHC class I chain-related protein A and B (MICA and MICB)-NKG2D ligands that are transiently upregulated on activated CAR-T cells. Preclinical evaluation showed an better expansion in vitro and an increase in anti-tumour activity compared to CYAD-01. First data indicate safety and tolerability but there are no efficacy data available yet (NCT04167696).[14]

CURRENT TARGETS

To achieve optimal results, the identification and selection of suitable targets, meeting multiple criteria, is imperative. Ideally, the expression of the target should allow the CAR-T cells to discriminate between leukemic stem cells (LSCs) and normal hematopoietic stem cells (HSCs). A high expression in LSCs as well as few or absent expression in HSCs, is the best scenario to accomplish maximal targeting with minimal side effects. A good

correlation between LSC load and prognosis is herewith evidently ideal.[15] Several targets for CAR-T cell treatment were investigated in AML and the best ones are discussed below:

The interleukin-3 receptor alpha chain (IL-3Rα), also known as **CD123**, is highly expressed in AML, acute lymphoblastic leukaemia (ALL), blastic plasmacytoid dendritic cell neoplasm, hairy cell leukaemia, and certain lymphomas, marking CD123 as an attractive target for CAR-T therapy. Importantly, it is also expressed on HSCs, regulating their proliferation and differentiation, and on normal endothelial cells, predicting high toxicity when targeting CD123 without adjustments.[16] Therefore, a rapidly switchable universal CAR-T platform (UniCAR, Figure 1) was developed in order to control and dose the activation of CAR-T, using an optimized CD123-specific targeting module (TM123).[17] This TM123 is a soluble adaptor consisting of an antigen-binding moiety (here, directed against CD123) which has been linked to a motif recognized by the UniCAR-T and allows control over the CAR-T reactivity. . Preclinical evaluation showed no difference between CD123-specific CAR-T and CD123 UniCAR-T in efficiently eliminating $CD123^{high}$ cells. Interestingly, modular CAR-T also kills weakly CD123-positive cells, but after TM123 withdrawal, these low expressing cells can recover. In contrast, the continuously active CD123 CAR-T slowly killed all the $CD123^{low}$ cells without the possibility to recover, resulting in more severe haematological toxicity.[17] These results indicate that although this switchable approach could lead to less myeloablation, as normal HSCs also have a low CD123 expression, premature deactivation of the UniCAR-T cells could be associated with escape $CD123^{low}$ leukemic blasts. Recently, a clinical trial with UniCAR-T cells and TM123 has started in patients with haematologic and lymphatic malignancies NCT04230265).

CD33 is a myeloid-specific sialic acid-binding receptor with expression in almost 90% of the AML patients. Because of its myeloid specificity, CD33 is an interesting target and over the past decade multiple anti-CD33 therapies, including monoclonal antibodies and bispecific T-cell engagers targeting CD33 have been developed.[18-20] Recently, anti-CD33 CAR-T cells were manufactured with promising preclinical data, leading to multiple ongoing clinical trials (Table 1). However, O'Hear and colleagues previously formulated a cautionary note on the expected profound myelosuppressive effect upon long-term persistence of CD33 CAR-T cells in vivo.[18]

Human C-type lectin-like molecule 1 (**CLL-1**, CLEC12A, MICL, KLRL1 or DCAL-2) is a type II transmembrane glycoprotein, functioning as an inhibitory receptor. The molecule is expressed in myeloid cells and AML blasts, as well as present on LSCs and absent on HSCs. Altogether, CLL-1 seems to be an excellent target for adoptive therapy. The efficacy of CAR-T therapy targeting CLL-1 has been tested preclinically and proven to decrease the AML cell load, without damaging normal HSCs.[21]

CD7 is a transmembrane glycoprotein, expressed by the leukemic blasts and HSCs of approximately 30% of AML patients. There is an association between expression and more aggressive disease and/or therapy resistance, making it an interesting target for adoptive therapy in this subset of patients.[22] Healthy T and NK cells also express CD7 predicting non-feasibility of CD7-directed CAR-T therapy, even though the function of CD7 in T cells was proven redundant using a knock-out mice model.[23] Therefore, *CD7*-edited CD7 CAR-T cells were developed and shown to selectively target and kill AML precursor cells and blasts *in vitro* and in a xenograft model. There was no toxicity observed towards normal myeloid cells and HSC.[22]

B7-H3 is a type I transmembrane protein, functioning as a negative regulator of T cells. It is highly expressed in multiple solid tumours and in haematological malignancies like AML, while being almost absent in healthy tissues. Preclinical evaluation of B7-H3-specific CAR-T cells illustrated significant antitumor activity towards AML cells in vitro as well as in xenograft mouse models.[24, 25] Clinical trials of B7-H3-targeted CAR-T cells in solid tumours are ongoing (NCT04483778), most of them in patients with glioblastoma (NCT04385173 and NCT04077866). Clinical trials are in progress for various advanced solid tumours (NCT03525782 and NCT02587689), but no trials in AML patients have started yet.

Next, mucin1 (**Muc1**) is a transmembrane glycosylated protein with a crucial role in leukaemia stem-cell function, that induces reactive oxygen species and promotes myeloid differentiation. Cell death in vitro and in vivo can be induced by targeting Muc1, making it an interesting target for adoptive therapy.[26] Furthermore, Muc1 is a very attractive target in other malignancies too, where Muc1-specific CAR-T cells have already been manufactured with promising first results in vitro and in xenograft models.[27-29]

CD117 or **c-KIT** is a cognate receptor tyrosine kinase for stem cell factor and is highly expressed on both normal HSCs and most of the AML cells. In vitro targeting with CD117-specific CAR-T cells showed efficient killing of healthy HSCs and leukemic cells, and eradication of the disease was demonstrated in vivo in CD117-positive AML xenografts.[30] Fine-tuned anti-CD117 CAR-T cells are promising to be used as an excellent myeloid conditioning therapy.[31]

Another promising target is the neural cell adhesion molecule 1 (**NCAM-1**), also known as **CD56**, with expression in almost 20% of all AML patients. NCAM-1 is an important regulator of neurogenesis, cell proliferation and migration, and there's a 53-fold higher expression in LSCs compared to normal HSCs. Although it is widely used for monitoring of MRD, the hematopoietic function remains elusive.[32] Furthermore, CD56 is linked to disease progression in many types of cancer and associated with drug resistance in AML.[33] Suppressing NCAM1 showed inhibition of cell growth and enhancement of differentiation and cell death. Taking all these features into account, NCAM-1 could not only serve as a biomarker to guide AML treatment, but its potential as a therapeutic target needs further exploration.[33]

Finally, **NKG2D** is a receptor expressed on NK cells and T cells and NKG2D ligands are upregulated in response to DNA damage, certain infections and mostly, in case of conversion to cancer. Various solid tumours and haematological malignancies express these ligands, with almost no expression on healthy tissues.[34]

CHALLENGING HURDLES

<u>The scarcity of specificity</u>

Considering no specific antigen has currently been identified in AML, collateral damage is inevitable and myeloablation can only be prevented through limiting CAR-T cell persistence. Creating a safety switch into the T cells by using a suicide gene or system, makes it possible to eliminate them in vivo when their persistence is no longer required.[8, 35] Subsequently, the co-expression of inducible caspase 9 (iCasp9) is currently under evaluation in a clinical trial in neuroblastoma (NCT01822652). Alternatively, incorporating a surface antigen in CAR-T cells and timely administration of exogenous antibodies allows destruction of the CAR-T cells when their anti-tumour activity is no longer required. Furthermore, using mRNA electroporation in the development of the CAR-T cells is also very promising, since the degradation of the mRNA innately reduces the CAR-T cell activity.[35]

Next to its specific expression, the antigen should preferentially have universal expression in all LSCs, whereby additional expression in the leukemic blasts could add to the chances of achieving complete remission. Taking into account the great inter- and intra-patient heterogeneity that characterizes AML, a next criterium should be the ability of the target to identify LSCs independently of this complicated heterogeneity. Ultimately, the expression pattern should be stable and should not vary between time of diagnosis and relapse.[15]

Finally, in order to facilitate the development of targeted therapy and CAR-T cells, protein expression on the cell surface would be more desirable. Some AML-specific markers, such as the T-cell receptor γ chain alternate reading frame protein (TARP), are known to be only expressed intracellularly, making them difficult to target since CAR-T cells usually recognize extracellular antigens that are expressed on the cell surface.[36] By using a T cell receptor (TCR)-mimic CAR (TCRmCAR, Figure 1), an intracellular onco-protein could be presented on the cell surface, allowing the CAR-T cells to target and eliminate cancer cells that express the intracellular

marker. These TCRmCARs integrate a a small chain variable fragment (scFv) that recognizes a portion of intracellular tumor-associated peptides in the context of human leukocyte antigens (HLAs). These new redirected CAR-T cells have not yet been tested clinically but suggest that an extracellular expression of the tumour-specific antigen may no longer be mandatory.[35, 37]

However, a long period of myeloablation is known to be associated with bleeding toxicity and neutropenic infections. Therefore, transplanting edited HSCs without the expression of the targeted antigen, allows for CAR-T cell persistence as well as normal haematopoiesis by the antigen-negative donor allograft. This idea has been tested for CD33-negative HSCs and CD33 CAR-T cells and proven to be effective in vitro and in xenograft models.[38, 39]

The complexity of CAR-T cell engineering

The intrinsic properties of the CAR-T cells are also very important for their successful application. The choice for a proper viral vector and in general the incorporation method of CARs into T cells, seems to be essential.[8] Furthermore, the quality of the T cells is dependent of the status of the patient at the moment of leukapheresis. Since many patients already underwent long and heavy therapy, this T cell quality is unfortunately often below par. The way to overcome this last issue is by either collecting T cells upfront to guarantee a better quality, or to use allogeneic T cells. With the latter option, one should take into account the risk for graft versus host disease (GvHD) which might be prevented through intensifying the pre-conditioning process or TCR depletion, which is being investigated in ongoing clinical trials (NCT03166876 and NCT03229876). Furthermore, the use of γδ T cells is very promising, as they are not alloreactive and have no risk for GvHD, in contrast to αβ T cells.[40] Lastly, the use of alternative immune effector cells, such as natural killer (NK), is a favourable method to reduce the risk for GvHD.[7, 35, 41]

The induced immunosuppression

The observed GvL phenomenon post-allo-HSCT proofs that AML is immunosensitive and that adoptive immune therapy is an efficient way to remove tumour cells. However, AML cells also secrete cytokines and induce co-inhibitory receptors on T cells to suppress and evade the immune system. [34, 35]

This understanding has led to development of therapeutic options to enhance CAR-T cell efficacy through blocking co-inhibitory receptors. The FDA approved various co-inhibitory receptor blockers (anti-CTLA-4, anti-PD-1, and anti-PD-L1) that are currently implemented in clinical practice and show promising results. Incorporating this blockade mechanism in CAR-T cells is up-and-coming. Other immune-mediating therapy to alter the escape from immunosurveillance are being investigated as well.[35, 42, 43]

CONCLUSION

The heterogenicity of AML, lack of specific target and the associated haematological toxicity, makes it difficult to implement adoptive therapy into AML clinical practice. Nevertheless, many ongoing preclinical and clinical studies deliver promising results and at least suggest that the success seen in B cell malignancies might be replicated in myeloid malignancies in the near future. A deep understanding of the AML biology and microenvironment and further improvements in CAR-T designs, will help us to optimize CAR-T cell therapy in AML, improve the clinical outcome of AML patients and reduce side effects.

KEY MESSAGES

- The field of immunotherapy in AML is rapidly evolving
- Multiple forms of CAR-T cell therapy are in clinical studies showing promising results
- Several hurdles still have to be overcome in the search for the holy grail

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AUTHOR DISCLAIMER

No financial support was received from any party in relationship with the content of this manuscript.

Table 1. Clinical trials with CAR-T cell therapy for acute myeloid leukemia (AML).

	Antigen Target	Clinical trials	Stage	Recruitment start	Published Results
Single targeting	CD123	NCT02159495	Phase I	2014	1 r/r AML-M2, blast reduction (59% to 45%)
		NCT03190278	Phase I	2017	
		NCT03766126	Phase I	2018	
		NCT04318678	Phase I	2020	
	Uni-CAR (TM123)	NCT04230265	Phase I	2020	
	CD33	NCT01864902	Phase I/II	2013	1 refractory AML, temporary blast reduction (>50% to <6%)
		NCT03126864	Phase I	2017	
		NCT03971799	Phase I/II	2019	
	CLL-1	NCT04219163	Phase I	2020	
	NKG2D: CYAD-01	NCT02203825	*Phase I*	2014	7 AML patients, no clinical efficacy ***(STOP announced)***
		NCT03018405	*Phase I/II*	2017	Temporary CR_h/CR_i in 3/7 r/r AML (ORR 42%) ***(STOP announced)***
	CYAD-02	NCT04167696	Phase I	2020	
	CD7	NCT04033302	Phase I/II	2019	
Dual targeting	CD123, CLL-1	NCT03631576	Phase II/III	2018	
	CLL-1, CD33 (cCAR)	NCT03795779	Phase I	2019	CR in 7 out of 8 r/r AML and 1 CML patient
Multi-targeting	CD33, CD38, CD56, CD123, Muc1, CLL-1	NCT03222674	Phase I/II	2017	

(r/r = relapsed and/or refractory, CR = complete remission, CR_h = complete remission with hematologic recovery, CR_i = complete remission with incomplete marrow recovery, ORR = overall response rate, CML = chronic myeloid leukaemia)