Myeloid Malignancies

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In addition to chemotherapy, which remains the basic treatment, the treatment panel for acute myeloid leukaemia (AML) has expanded considerably in recent years. Clinicians now have a large choice of therapies: targeted therapies (anti-IDH1/2, anti-FLT3, and anti-BCL2 therapies, among others), drugs targeting epigenetic mechanisms, kinase inhibitors (FLT3, MAPK, and JAK2, etc.), immunotherapies (monoclonal antibodies linked or not to a toxin, dual/bispecific), and cellular immunotherapies. Moreover, despite its toxicities, allogeneic transplantation often remains an effective final therapeutic alternative. However, most patients are refractory or relapsed (R/R) after several lines of therapy. Thus, there is a clinical need in AML R/R patients, and CAR-T cells may be an option and can find a place in the treatment to reduce tumour burden and clinical evolution of the disease (Fig. 18.1, modified from Roussel et al. (2020)).

Several currently ongoing research programs aim to generate CAR-T cells against myeloid malignancies (Hofmann et al. 2019). However, the absence of a truly AML-specific marker generates remarkable uncertainty regarding the optimal antigens to target, and significant concern remains about off-target effects on normal haematopoiesis. The difficulty of obtaining successful manufacture of CAR-T cells from heavily pretreated patients has paved the way to investigation of different cell sources to build alternative platforms for cellular therapy.

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N. Kröger et al. (eds.), *The EBMT/EHA CAR-T Cell Handbook*,
https://doi.org/10.1007/978-3-030-94353-0_18

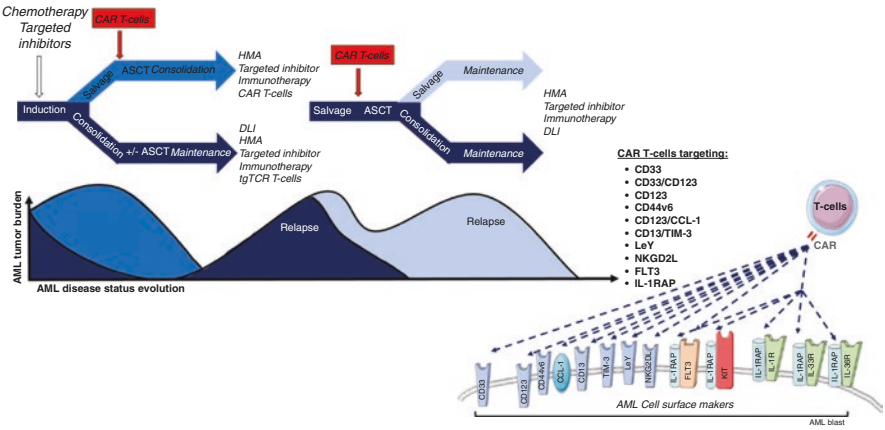

Fig. 18.1 Putative place of CAR-T cells in the AML treatment strategy. *HMA* hypomethylated agent, *DLI* donor lymphocyte infusion, *tgTCR* transgenic T cell receptor T cells, *ASCT* allogeneic stem cell transplantation

Single or Dual Antigen Targeting?

CAR-T cells targeting CD33 and CD123 have already been investigated in early phase clinical trials. Unfortunately, these antigens do not avoid "on-target off-tumour" effects, such as myelotoxicity and endothelial toxicity. For this reason, CAR-T cells directed against other surface proteins, such as CCL-1, CD44v6, FLT3, c-KIT (CD117), CD38, B7-H3 (also known as CD276), NKG2D, and IL-1RAP, are also under preclinical and clinical investigation (Table 18.1).

CD123 CAR-T cells induce haematopoietic toxicity but on a smaller scale than CD33 CAR-T cells, particularly following anti-CD123 single chain fragment variable (scFv) modifications (Mardiros et al. 2013; Gill et al. 2014; Thokala et al. 2016). Nevertheless, based on their expression on stem cells, CD123 CAR-T cells could be used as a myeloablative regimen before ASCT, thus representing an interesting strategy for treatment of R/R AML patients (Gill et al. 2014; Cummins and Gill 2019; Testa et al. 2019). Notably, IL-15 may enhance the anti-AML activity of CD123 CAR-T cells (Mu-Mosley et al. 2019). Targeting FLT3 or CD117 could be an attractive option, again in association with ASCT (Jetani et al. 2018; Myburgh et al. 2020). Targeting of the Lewis Y antigen and NKG2DL CAR-T cells has also been proposed, but phase 1 trials have shown short response durations, despite reduced toxicity (Ritchie et al. 2013; Driouk et al. 2019). CAR-T cells targeting CD44v6 mediate potent antitumour effects against AML while sparing normal haematopoietic stem cells (Casucci et al. 2013), and a clinical trial is currently ongoing. A potent effect on LSCs was observed with CAR-T cells targeting IL1RAP (Warda et al. 2019) with no apparent effect on healthy haematopoietic stem cells. Similar more specific antileukaemic activity was observed by targeting FLT3 and KIT mutations (Mitchell et al. 2018). Interestingly, targeting IL1RAP decreases IL-1, IL-6, IL-10, IL-13, IL-17, IL-22, IFNγ, and TNFα levels (Højen et al. 2019). The

Table 18.1 CAR-T cell immunotherapies under investigation in AML (based on www.clinicaltrials.gov at 05/25/2020)

CAR-T cells	Preclinical results	Status	Clinical trials
CD33	Myeloablative, ASCT requirement	Phase 1	NCT03126864
		Phase 1/2	NCT03971799, NCT01864902
CD123	Myeloablative, ASCT requirement	Phase 1	NCT03796390, NCT03585517, NCT03114670, NCT03766126, NCT04014881, NCT03190278, NCT02159495, NCT04230265, NCT04318678, NCT03672851
		Phase 1/2	NCT04272125, NCT04265963, NCT04109482, NCT03556982
CCL-1	AML and HSC targeting	Phase 1	NCT04219163
CD38	AML targeting	Phase 1/2	NCT04351022
CD44v6	AML targeting	Phase 1/2	NCT04097301
FLT3	Myeloablative, ASCT requirement	Phase 1	NCT03904069
KIT (CD117)	Myeloablative, ASCT requirement	Preclinical	NCT03473457
B7-H3	HSC toxicity reduction	Preclinical	None
CD13 TIM-3	HSC toxicity reduction	Preclinical	None
PD-1	Antitumour enhancement	Preclinical	None
Lewis Y	Short duration of response, few toxicities	Phase 1	*NCT01716364, no further study*
NKGD2L	Short duration of response, few toxicities	Phase 1	*NCT02203825, no further study*
IL1RAP	LSC targeting	Preclinical	NCT04169022
CD33/CD123	AML and HSC targeting	Phase 1	NCT04156256
CCL-1/CD123	AML targeting	Phase 2/3	NCT03631576
CCL-1/CD33	AML targeting	Phase 1	NCT03795779
CCL-1/CD33 and/or CD123	AML targeting	Phase 1/2	NCT04010877
Muc1/CLL1/ CD33/CD38/ CD56/ CD123	AML targeting	Phase 1/2	NCT03222674

Studies investigating T cell immunotherapies in AML. *AML* acute myeloid leukaemia, *LSC* leukaemic stem cell, *HSC* haematopoietic stem cell, *ASCT* allogeneic stem cell transplantation

reduced production of IL-4, IL-6 and IL-10 and absence of IL-17 production (Warda et al. 2019) may in turn limit CAR-T cell cytokine release syndrome (CRS) and the immune effector cell-associated neurotoxicity syndrome (ICAN) associated with excessive production of IL-1 (Garcia Borrega et al. 2019). Notably, the reduction in IL-1β, IL-6, and TNFα levels leads to decreased release of IL-10 and TGFβ, which impair CAR-T cell functions (Epperly et al. 2020).

CAR-T cells simultaneously targeting CD33 and CD123 are also in development and exhibit pronounced antileukaemic activity (Petrov et al. 2018). Similarly, CD123 and CCL-1 compound CAR-T cells may be useful for active targeting of leukaemia stem cells (LSCs) (Morsink et al. 2018; Shang and Zhou 2019).

Bispecific CD13-TIM-3 CAR-T cells (He et al. 2020) and B7-H3 CAR-T cells (Lichtman et al. 2018) showed reduced HSC toxicity. Moreover, the B7-H3 pan-cancer target was also studied in solid tumours (Waldman et al. 2020). Preliminary reports show that PD-1 inhibitors also regulate the CAR-T cell response, although few data are available. Furthermore, delivery of PD-1-blocking scFv CAR-T cells in preclinical investigations demonstrated interesting antitumour efficacy enhancement (Anonymous 2019). Several challenges remain to be overcome, as recently reported, and further investigations may provide a better understanding (Mardiana and Gill 2020).

Molecular Engineering of the Chimeric Receptor and Alternative Cell Sources

Beyond the selected target, optimizing the molecular engineering of the chimeric receptor remains crucial. CD33 4-1BBz CAR-T cells have shown antileukaemic activity and resistance to exhaustion with increasing central memory comportment (Li et al. 2018). An additional strategy that has been proposed to reduce haematopoietic toxicity is the use of a transiently expressed CART33 to induce self-limiting activity against AML cells (Kenderian et al. 2015). Another proposed strategy is to inactivate the *CD33* gene in HSCs prior to transplantation to prevent CD33-induced haematopoietic toxicity of CAR-T cells (Kim et al. 2018).

In addition, to avoid or reduce the uncontrolled toxicity of expanding CAR-T cells, the use of the anti-CD52 antibody alemtuzumab or a suicide gene strategy based on CD20 protein coexpression in CD123 CAR-T cells has been proposed for subsequent anti-CD20 targeting with rituximab (Introna et al. 2000; Tasian et al. 2017).

Several clinical trials are currently evaluating the use of allogeneic CAR-T cells in haematologic malignancies, employing different effector cell types, such as NK cells (Daher and Rezvani 2021) or TCR-edited cells (Provasi et al. 2012), to limit GvHD and develop strategies to avoid the rejection of allogeneic cells. In this regard, the limited GvHD associated with the use of cytokine-induced killer (CIK) cells (Martino Introna et al. 2017) was confirmed in a phase I/IIa study in which B-ALL patients who relapsed after allogeneic transplantation were treated using CD19-specific CAR CIK cells (CARCIK-CD19) manufactured from a previous transplant donor (Magnani et al. 2020). Notably, this study provides evidence of the feasibility of employing a nonviral sleeping beauty transposon system to successfully produce CARCIK cell products starting from a small amount of donor-derived PB, thus offering a valid alternative to viral vectors. The use of CAR-engineered CIK cells was also demonstrated to be effective for AML by characterizing the targeting of the two most validated AML molecules, CD33 and CD123, in vitro and in vivo (Tettamanti et al. 2013; Pizzitola et al. 2014; Arcangeli et al. 2017; Rotiroti et al. 2020).

Key Points

- The primary challenge limiting the use of CAR-T cells in myeloid malignancies is the absence of an ideal antigen.
- Myeloid antigens are often coexpressed on normal haematopoietic stem/progenitor cells (HSPCs).
- Myelotoxicity and endothelial toxicity can be overcome by "and/or" dual CAR targeting.
- Allogeneic CAR-T cells may be a future alternative.

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