short review

memo (2020) 13:50–54
https://doi.org/10.1007/s12254-020-00577-1

memo
magazine of european medical oncology

AML—is it time to drive a CAR(-T)?

Jakob D. Rudzki · **Dominik Wolf**

Received: 30 November 2019 / Accepted: 12 January 2020 / Published online: 5 February 2020

Summary The treatment options for newly diagnosed and relapsed/refractory acute myeloid leukemia (AML) have substantially improved over the last 5 years. However, even though novel targeted agents (e.g. venetoclax, IDH1/2 and novel FLT-3 inhibitors; cytosolic isocitrate dehydrogenase 1/2 and fms-like tyrosine kinase 3 inhibitor) and improved chemotherapeutics (e.g. CPX-351; liposomale Daunorubicin/Cytarabine) are entering clinics, physicians are still confronted with high relapse and treatment failure rates. Thus, novel new strategies are required to improve AML therapy. Application of genetically engineered T cells (i.e. chimeric antigen receptor T cells, CAR-T cells) has proven to be highly effective in B cell-derived neoplasia and early data suggest also a high potential in the treatment of AML. This short review highlights the current approaches but also limitations of CAR-T cell therapy in AML precluding their current routine clinical use. Among a plethora of problems to be overcome, a critical issue will be to find relatively selective actionable targets in AML.

Keywords Relapsed/refractory AML · CD123 · CD33 · CD135 · TCR-C4

CAR transgenic T cells have led to impressive clinical results in treating patients with relapsed and refractory B cell leukemia and lymphoma [1–3]. To date, increasing numbers of publications are reporting trials using CAR transgenic T cells in B cell-derived malignancies. Most of these strategies are directed against CD19 as target antigen, a B cell-lineage antigen expressed on the surface of normal as well as on many malignant B cells (e.g. B-NHL and acute lymphoblastic leukemia). Long-term follow-up of up data now reaching 3 years revealed convincing results in chemotherapy refractory or relapsed DLBCL patients [2–4].

Similar to CD19 targeting also additional or alternative target antigens, such as CD20 are approached as target for CAR T cell therapy in B cell malignancies. Early studies illustrate modest efficacy without significant toxicities suggesting that CD20 targeting may possibly be an additional target on B-NHL [5]. Recently, a phase IIa clinical trial reported an objective response rate (ORR) of 82% (complete remission [CR] of 6/11 and partial remission [PR] of 3/11) with well-tolerated toxicity [6]. These early promising findings suggest that CD20 targeting may represent an interesting additional or alternative target, for example, by applying dual or bispecific CAR-T cells targeting both CD19 and CD20.

AML is the most common acute leukemia in adults. Even though many drugs were approved during the last 2 years, the disease is still characterized by a very poor prognosis reflected by only a minority of patients achieving long-term survival. The overall cure rate in AML patients below 60 years is about 35–40% and drops to 5–15% for patients older than 60 years. Patients who never reach remission or suffer from early relapse within 6 months of CR (complete remission) are less likely to respond to any other salvage treatment [7]. Thus, high primary treatment failure and frequent AML relapse remains a significant problem in the clinical management of AML. Since the availability of hypomethylating agents (HMA) and more recently

Dr. J. D. Rudzki (✉) · D. Wolf
Internal Medicine V, Medical University of Innsbruck, Anichstraße 35, 6020 Innsbruck, Austria
jakob.rudzki@tirol-kliniken.at

D. Wolf
Medical Clinic III, Oncology, Hematology, Immuno-Oncology and Rheumatology, University Clinic Bonn, Sigmund-Freud-Straße 25, 53127 Bonn, Germany

also of venetoclax, AML therapy in patients unfit for intensive induction has improved, even though it remains a palliative setting. Thus, apart from AML M3 and ELN good risk AML (in both of which consolidation is drug-based), allogeneic stem cell transplantation has been the most frequently used consolidation strategy in intermediate- or high-risk AML patients fit for transplant and having a suitable donor. However, the latter consolidation is still characterized by high relapse rates as well as significant treatment-related mortality, which underscores the high medical need to improve AML management. Cell-based treatment strategies using CAR-T cells thus represents an appealing approach to be tested also in AML patients, but the limited availability of appropriate targets still represents a major obstacle for a broader success of this cell-based treatment concept in AML.

Effectiveness of CAR-T cell treatment depends on the choice of the optimal target

The extracellular domain of the transgenic CAR construct recognizes a predefined target on the respective leukemia/tumor cells. Upon target binding, the chimeric construct containing intracellular co-stimulatory signaling domains, such as 4-1BB or CD28 connected to CD3zeta [8, 9], potently activate the CAR-T cell leading to target cell killing and stimulation of other immune cells in the adjacent tumor microenvironment.

Identifying the optimal target in the case of AML still remains a big challenge. Owing to the positive results of anti CD33-directed therapy with the immunotoxin gemtuzumab ozogamizin, CD33-directed therapies represent a promising tool, particularly because almost 90% of AMLs express CD33 [10]. Moreover, 70% are marked by being double expressors of CD33 and CD123 and only 10% express CD123 without concomitant CD33 on their surface [11] making both CD33 together with CD123 interesting targets for CAR-T in AML. However, based on expression of these markers also on non-leukemic hematopoietic stem cells, dual targeting [12, 13] of CD33 together with CD123 not only eradicated the leukemia, but also caused long-term myeloablation that required subsequent HSCT (hematologic stem cell transplantation) to allow for hematopoietic recovery. Several other target antigens, like CD174 (Lewis Y antigen), CD44v6, CLL-1 (CLEC12A), CD7, folate receptor beta (FRbeta), and CD135 (FLT-3 receptor) were subsequently defined allowing a more selective targeting of AML. Unfortunately, none of the mentioned target molecules is exclusively expressed on AML blasts as most of them are also detectable on different hematologic progenitor cells or other healthy tissues (keratinocytes, neurons), thereby limiting the clinical translation [12].

In addition, another hurdle of CAR-T cell therapy is the clonal heterogeneity of the AML. In this respect, dual targeting, as already tested in B-ALL CAR-T trials, may help to overcome this issue, but still elimination of healthy stem cells remains an issue [6, 13]. This may also help to prevent treatment failure mediated by antigen loss, a phenomenon well described for CD19-expressing ALL. Another resistance mechanism is a structural change of the target molecule induced by selection of mutated subclones [14]. Similar to antigen loss, this escape mechanism can at least be overcome by dual targeting concepts.

The clue is to find well expressed antigen sets represented on AML blasts but excluding healthy hematopoietic cells and non-hematologic tissue. Perna et al. integrated large transcriptomics and proteomics datasets from malignant and normal tissue and developed an algorithm to identify potential targets expressed on leukemic stem cells. Their findings identified potential targets expressed in leukemia cells but not in normal $CD34^{+}CD38^{-}$ hematopoietic cells, T cells or vital tissues which therefore could serve as novel promising CAR-T targets [15].

Clinical status of CAR-T therapy in AML

The first clinical CAR-T trial in AML was developed based on the observation that the Lewis Y (LeY) oligosaccharide is overexpressed on many epithelial cancers as well as hematologic malignancies (including AML) with limited expression on healthy tissues (Clinical Trials GovNo.: NCT01716364). A 2^{nd} generation CAR-T cell product was tested in 4 patients with relapsed AML upon preconditioning with chemotherapy. Two patients achieved long-term remission, one patient a cytogenetic remission and the fourth patient showed a significant reduction of circulating blasts. The most notable finding from this study however was the lack of significant toxicity and the durable in vivo persistence of the CAR-T effectors [16]. LeY was the first antigen that was successfully implemented in CAR-T cell therapy approaches in AML (Table 1).

Another research group focused on CD38 as target. In this study the investigators could reveal another example of ATRA-enhanced cytotoxicity by using CD38-41-BB-CD3zeta CARs. Those results highlighted that ATRA may be an interesting combinatorial partner for CAR-T cells in AML [17]. ATRA is widely used for the treatment of acute promyelocytic leukemia (APL). It induces the differentiation of APL -cells, leading to suppression of proliferation capacity of these cells. Beside these effects, it has been reported that it may enhance CD38 expression on AML blasts [18].

As already mentioned above, CD33 is a highly interesting target as it is expressed in almost all AML patients as well as in AML stem cells. Because of these facts, CD33 is a promising CAR-T target. A trial by M. Kim et al. proposed an interesting but ambitious approach by CRISPR/Cas9-mediated removal of CD33 from normal hematopoietic stem and progenitor cells, thereby rendering these cells resistant to CD33-targeted CAR-T therapy [19]. Patients with min-

Table 1 The main evolution of CAR-Ts in AML (adapted from [22])

History of main types of CAR-Ts in AML				
Year				
2010	t: Lewis Y 2ndG:CD28+CD3zeta	t: CD33 CIK	–	–
2011	t: CD33 EBV-CTL	–	–	–
2013	t: CD123 CIK	t: Lewis-Y 2ndG:CD28+CD3zeta	t: CD123 2ndG:CD28+CD3zeta	t: CD44v6 2ndG:CD28+CD3zeta +suicide gene iC9
2014	t: CD123 2ndG:4-1BB+CD3zeta	t: CD123 2ndG:CD28+CD3zeta+EGFRt	t: NKG2DL 2ndG:DAP10+CD3zeta	t: FRbeta 2ndG:CD28+CD3zeta
	Comparing the efficacy & safety of CD33-CIK & CD123-CIK	t: CD33 2ndG:4-1BB+CD3zeta	–	–
2015	t: CD33 CD137+TCR	t: CD123 2ndG:4-1BB +TCR	–	–
2016	t: NKG2DL completed	t: CD33 – allogeneic ph1	t: CD38 2ndG:4-1BB +CD3zeta +ATRA	t: CD33 2ndG:CD28+0X40+ CD3zeta +suicide gene iC9
↓	"GoCAR-T" CD123+iMC costimulation	t: CD33 2ndG:CD28+CD3zeta+EGFRt	t: FLT3 2ndG:4-1BB +CD3zeta	t: CD123 – DART
	–	–	–	t: CD7
	–	–	–	t: CLEC12A

t target, *2nd G* second generation, *CIK* cytokine induced killer, *EBV-CTL* Ebstein Barr Virus-cytotoxic lymphocyte, *NKG2DL* natural killer group 2D ligand, *EGFRt* a tag derived from epidermal growth factor receptor, is the antigen of the clinically available antibody cetuximab, *DAP10* a type of natural adaptive protein, provides a costimulatory signal similar to that of CD28, *ATRA* all trans retinoic acid, a drug that up-regulates the expression of the target antigen, resulting in improved anti-leukemic activity, *GoCAR-T* a structure comprising a proliferation-deficient first generation CAR and a ligand-dependent activation switch (e.g. iMC) that efficiently eradicates CD123+AML cells when co-stimulated with systemic rimiducid administration, *iMC* inducible MyD88/CD40 is a ligand (rimiducid)-dependent co-stimulatory switch, *DART* dual-affinity re-targeting, *TCR* T cell receptor, *CLEC12A* C-type lectin domain family 12 member A.

imal residual disease of AML first undergo conditioning before receiving allogeneic donor-derived CD33 KO HSPCs (CD33 knocked out hematopoietic stem and progenitor cells) followed by administration of CD33-directed CAR-T from the same donor.

Similar to CD33, the transmembrane alpha chain of the interkleukin-3 receptor, CD123, is expressed on AML blasts to a high extent whereas it displays lower expression levels on healthy hematopoietic (stem) cells. Two clinical trials validated the effect and the safety profile (NCT02159495 and NCT02623582) of CD123-directed CAR-T. Concerns of a potential myeloablative effect by removing $CD123^+$ cells could not be verified. One group even generated a 4th generation CAR-T (antiCD123-CD28-CD137-CD27-CD3zeta-iCASP CAR = 4SCAR123) that is characterized by potent cytotoxicity against AML blast cells in vitro and then administered those CAR-Ts to a 47-year-old man with AML M2 (presented at ASH 2015 abstract #3778: First in man CD123-specific chimeric antigen receptor-modified T cells for the treatment of refractory acute myeloid leukemia). Advanced protocols proposed to combine fully myeloablative CD123 CAR-Ts in conjunction with an adjacent allogeneic stem cell transplantation (SCT) to reduce the risk for AML relapse; however clinical data are not yet available.

FLT3 (Fms-like tyrosine kinase 3) also known as CD135 is a cytokine receptor belonging to the class III receptor tyrosine kinases. This gene belongs to the most commonly mutated genes in AML, characterized by internal tandem duplications of FLT3 (FLT3-ITD) reaching about 25% of AML cases. Targeting FLT3 by using a 2nd generation CAR (4-1BB-CD3zeta) demonstrated potent anti-AML activity. Jetani et al. even demonstrated synergistic activity with a FLT3 inhibitor. Concomitant administration of crenolanib leads to an increase of surface expression of FLT3 specifically on $FLT3\text{-}ITD^+$ AML cells and consequently, enhanced recognition by FLT3-CARTs *in vitro* and *in vivo*. As anticipated, the myeloablative effect on healthy hematopoietic cells *in vitro* and *in vivo* required subsequent CAR-T depletion and allogeneic transplantation to reconstitute the hematopoietic system [20], again underscoring the current problem of limited AML-selectivity of the described CAR-T strategies.

Based on phase 1 data presented on the ASH 2017 and the American Association for Cancer Research (AACR) Special Conference on Tumor Immunology and Immunotherapy in November 2018 Mustang Bio initiated a single-center, first in human phase 1 dose-escalation clinical trial (City of Hope; NCT02159495) evaluating the safety and anti-tumor activity of escalating doses of MB-102 (CD123 CAR-T) in patients with relapsed or refractory AML (cohort 1) and BPDCN (blastic plasmacytoid dendritic cell neoplasm, cohort 2). They demonstrated complete responses at low

doses in BPDCN without dose-limiting toxicities. Dose escalation continues at City of Hope for both indications. In December 2018 Mustang Bio received Orphan Drug Designation for MB-102 for the treatment of BPDCN, a rare and incurable blood cancer with a median survival of less than 18 months and no standard of care. The great surprise came in July 2019 where FDA granted orphan drug designation to MB-102 for patients with acute myeloid leukemia. Subsequently, M. Litchman, president and CEO of Mustang Bio, announced to launch a phase I/II multicenter trial for patients with AML, BPDCN and high-risk MDS (myelodysplastic syndrome).

A different but promising cellular approach in the allogeneic transplant setting is illustrated by Chapuis et al. and published recently in *Nature Medicine* in 2019 [21]. The investigators isolated a high-affinity Wilms tumor antigen 1-specific TCR (TCR_{C4}) from HLA-A2$^+$ regular donor repertoires, inserted TCR_{C4} into Epstein–Bar virus-specific donor CD8$^+$ T cells ($T_{TCR\text{-}C4}$) to minimize graft-versus-host disease risk and enhance transferred T cell survival. Those cells were prophylactically infused post HCT into 12 patients (NCT01640301). At a median of 44 months post infusion, relapse-free survival reached 100% compared to a concurrent group of 88 patients with a similar risk AML with a relapse-free survival of 54% ($p = 0.002$). The authors finally stated that these long-term persisting cells maintained TCR_{CR4} expression by staying polyclonal. This strategy appears prospective for preventing AML recurrence in individuals at increased risk of post-HCT relapse. It represents a different strategy beside CAR-Ts but highlights a sophisticated cellular treatment approach.

Take Home Message

Despite various approvals during the last 2 years, AML still represents a malignant disease with a dismal prognosis. This is reflected by many cases of primary or secondary refractory AML patients leading to relapse, even upon allogeneic stem cell transplantation. Even though CAR-T cell therapy is highly effective in NHL and ALL, CAR-T therapies in AML are limited by the insufficient selectivity of the currently approached CAR-T targets. Despite that, preliminary data are at least in part promising in terms of their anti-AML efficacy, but the potential elimination of healthy hematopoietic stem cells may lead to long-term myelosuppression requiring rescue allogeneic SCT. This currently limits the clinical development of this treatment strategy in AML and still claims for new investigational approaches.

Funding Open access funding provided by University of Innsbruck and Medical University of Innsbruck.

Conflict of interest J. D. Rudzki declares that he has received honoraria and speaker's fee from BMS, Roche, MSD, AstraZeneca, Amgen, Gilead-kite, Novartis and served as advisor for BMS, Roche, MSD, AstraZeneca, Amgen, Gilead-kite, Novartis and BMS/Celgene. D. Wolf declares that he has received honoraria and speaker's fee from Novartis, Gilead, BMS/Celgene, served as advisor for Novartis, Gilead, BMS/Celgene and GEMoaB and receives research funding from Novartis and BMS/Celgene.

References

1. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378:439–48.
2. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med. 2018;380:NEJMoa1804980–56.
3. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med. 2017;377:2531–44.
4. Crump M, Neelapu SS, Farooq U, Neste E, Kuruvilla J, Westin J, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. Blood. 2017;130:1800–8.
5. Wang Y, Zhang W, Han Q, Liu Y, Dai H, Guo Y, et al. Effective response and delayed toxicities of refractory advanced diffuse large B-cell lymphoma treated by CD20-directed chimeric antigen receptor-modified T cells. Clin Immunol. 2014;155:160–75.
6. Zah E, Lin M-Y, Silva-Benedict A, Jensen MC, Chen YY. T cells expressing CD19/CD20 bispecific chimeric antigen receptors prevent antigen escape by malignant B cells. Cancer Immunol Res. 2016;4:498–508.
7. Breems DA, Putten W, Huijgens PC, Ossenkoppele GJ, Verhoef G, Verdonck LF, et al. Prognostic index for adult patients with acute myeloid leukemia in first relapse. J Clin Oncol. 2005;23:1969–78.
8. Carpenito C, Milone MC, Hassan R, Simonet JC, Lakhal M, Suhoski MM, et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. Proc Natl Acad Sci USA. 2009;106:3360–5.
9. Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol Ther. 2009;17:1453–64.
10. Castaigne S, Pautas C, Terré C, Raffoux E, Bordessoule D, Bastie J-N, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid

leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. Lancet. 2012;379:1508–16.
11. Hwang K, Park C-J, Jang S, Chi H-S, Kim D-Y, Lee J-H, et al. Flow cytometric quantification and immunophenotyping of leukemic stem cells in acute myeloid leukemia. Ann Hematol. 2012;91:1541–6.
12. Haubner S, Perna F, Köhnke T, Schmidt C, Berman S, Augsberger C, et al. Coexpression profile of leukemic stem cell markers for combinatorial targeted therapy in AML. Leukemia. 2019;33:64–74.
13. Ruella M, Barrett DM, Kenderian SS, Shestova O, Hofmann TJ, Perazzelli J, et al. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. J Clin Invest. 2016;126:3814–26.
14. Sotillo E, Barrett DM, Black KL, Bagashev A, Oldridge D, Wu G, et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov. 2015;5:1282–95.
15. Perna F, Berman SH, Soni RK, Mansilla-Soto J, Eyquem J, Hamieh M, et al. Integrating proteomics and transcriptomics for systematic combinatorial chimeric antigen receptor therapy of AML. Cancer Cell. 2017;32:506-519:e5.
16. Ritchie DS, Neeson PJ, Khot A, Peinert S, Tai T, Tainton K, et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. Mol Ther. 2013;21:2122–9.
17. Yoshida T, Mihara K, Takei Y, Yanagihara K, Kubo T, Bhattacharyya J, et al. All-trans retinoic acid enhances cytotoxic effect of T cells with an anti-CD38 chimeric antigen receptor in acute myeloid leukemia. Clin Transl Immunology. 2016;5:e116.
18. Drach J, Zhao SR, Malavasi F, Mehta K. Rapid induction of CD38 antigen on myeloid leukemia cells by all trans-retinoic acid. Biochem Biophys Res Commun. 1993;195:545–50.
19. Kim MY, Yu K-R, Kenderian SS, Ruella M, Chen S, Shin T-H, et al. Genetic inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for acute myeloid leukemia. Cell. 2018;173(9):1439–1453.e1.
20. Jetani H, Garcia-Cadenas I, Nerreter T, Thomas S, Rydzek J, Meijide J, et al. CAR T-cells targeting FLT3 have potent activity against FLT3–ITD+ AML and act synergistically with the FLT3-inhibitor crenolanib. Leukemia. 2018;32:1168–79.
21. Chapuis AG, Egan DN, Bar M, Schmitt T, McAfee MS, Paulson KG, et al. T cell receptor gene therapy targeting WT1 prevents acute myeloid leukemia relapse post-transplant. Nat Med. 2019;25:1064–72.
22. Fan M, Li M, Gao L, Geng S, Wang J, Wang Y, et al. Chimeric antigen receptors for adoptive T cell therapy in acute myeloid leukemia. J Hematol Oncol. 2017;10:151.