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Anti-CD19 chimeric antigen receptor targeting of CD19 + acute myeloid leukemia

Gina Ma[a,1], Yi Wang[b,1], Tahmeena Ahmed[b], Ann-Leslie Zaslav[b], Laura Hogan[c], Cecilia Avila[d], Masayuki Wada[a], Huda Salman[e,*]

[a] iCell Gene Therapeutics LLC, Research & Development Division, Long Island High Technology Incubator, 25 Health Science Drive, Stony Brook, NY 11790, United States
[b] Department of Pathology, Stony Brook Medicine, Stony Brook, NY 11794, United States
[c] Department of Pediatrics, Stony Brook Children's Hospital, Stony Brook University Medical Center, Stony Brook, NY 11794, United States
[d] Department of OB-GYN, Stony Brook Medicine, Stony Brook University Medical Center, Stony Brook, NY 11794, United States
[e] Department of Internal Medicine, Stony Brook Medicine, Stony Brook University Medical Center, Stony Brook, NY 11794, United States

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ABSTRACT

Aberrant expression of CD19 in acute myeloid leukemia (AML) is commonly associated with t(8;21)(q22;q22), although AML cases lacking this translocation occasionally express CD19. Mixed-phenotype acute leukemia also frequently expresses CD19. Chimeric antigen receptor (CAR) technology is a major breakthrough for cancer treatment, with the recent approval of CD19-directed CAR (CD19CAR) for treating B-cell malignancies. However, little information exists on using CD19CAR for other CD19 positive neoplasms such as AML. Our findings indicate that CD19CAR therapy can potentially be used for those with mixed phenotype leukemia and a subset of AML cases.

1. Introduction

Recently, the FDA approved a CD19-directed CAR for treating B-cell malignancies. AML is a rapidly progressing malignancy that accounts for about 15–20% of acute childhood leukemias and 80% of acute adult leukemia cases [1]. Approximately 60–70% of AML patients relapse after treatment, and the five-year survival rate for adult AML is 27%. Regarding CAR, there is very little data on its application to AML treatment, and most pertains to clinical trials.

Mixed-phenotype acute leukemia is a rare form of acute leukemia, accounting for 2–5% of all cases of acute leukemias [2]. Several studies have shown poor survival outcomes for adult patients with biphenotypic leukemia when compared to matched controls to AML or ALL [3–5]. One type, mixed-phenotype acute leukemia with t(v;11q23); MLL rearranged, has a poor prognosis and primarily affects children. Mixed-phenotype acute leukemia remains a challenge to treat due to the difficulty of identifying whether the origin of leukemia is lymphoid or myeloid. This ambiguity has impacted the decision of whether to use lymphoid or myeloid treatment regimens. Mixed-phenotype acute leukemia may express relatively high levels of CD19, which can be targeted using a CD19CAR regardless of cell origin.

In this study, we retrospectively searched for AML expression of CD19 in patients at Stony Brook University Hospital and gathered data from 1/1/2012–10/20/2017 that included AML with aberrant expression of CD19 and mixed-phenotype acute leukemia. We also analyzed a sample taken from a patient with refractory AML to demonstrate the robust anti-tumor effects of CD19CAR *in vitro*.

2. Materials and methods

Donor T-cells were taken from residual samples following the protocol approved by the Institutional Review Board. The CD19bCAR construct was subcloned into a CAR lentiviral vector containing the same backbone described previously [6–8]. Lentiviral generation and co-culture killing assays were as described previously [6–8].

3. Results

We identified 527 AML cases from 1/1/2012–10/20/2017 and found overall CD19 expression in our AML cases to be 3.2%, with 17 out of 527 AML patients expressing CD19 (Table 1). The M2 subtype most commonly expressed CD19 in 4 out of 49 patients (8.1%); followed by the M4/5 subtype in 1 out of 25 patients (4%); M0/1 subtype in 2 out of 72 patients (2.7%); and AML, NOS in 8 out of 361 patients

* Correspondence to: Stony Brook Medicine, 101 Nicolls Road, Stony Brook, NY 11794, United States.
E-mail address: huda.salman@stonybrookmedicine.edu (H. Salman).
[1] These authors contributed equally to this work.

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Table 1
CD 19 positive acute myeloid samples from 1/1/2012–10/20/2017.

Acute myeloid leukemia subtype	CD19 positive
AML, NOS[a]	8/361 (2.2%)
AML, M0/1	2/72 (2.7%)
AML, M2	4/49 (8.1%)
AML, M3	0/17 (0%)
AML, M4/5	1/25 (4%)
Mixed-phenotype acute leukemia	2/3 (66%)
Overall	17/527 (3.2%)

[a] NOS based on flow cytometry only, not morphology. Flow cytometry generally is unable to subclassify leukemia, leading to higher cases of NOS.

(2.2%). Expression of CD19 was not found in the 17 cases of M3 AML. CD19 was expressed in 2 out of 3 mixed-phenotype acute leukemia patients (66%).

We developed a CD19 redirected chimeric antigen receptor T-cell, CD19b-CAR, using T cells isolated from a normal donor (Fig. 1A). The CD19b-CAR construct contains a leader sequence, anti-CD19-single chain variable (scFv) sequence, hinge domain, transmembrane domain, co-stimulatory domain (4–1BB) and intracellular signaling (CD3zeta). To create CD19b-CAR T-cells, human peripheral blood T-cells were transduced with the CD19b-CAR. After 48 h treatment with CD19b-CAR-lentiviruses, flow cytometry was utilized, determining surface expression of the CD19b-CAR on the T-cell surface to be 52% (Fig. 1B).

Cells of interest were freshly taken from the bone marrow of a 57-year-old woman diagnosed with acute myeloid leukemia M2 with FLT-3 mutation. Flow cytometry analysis revealed aberrant expression of CD7 and CD19. Cytogenetic examination showed a normal female karyotype and absence of the (8;21) translocation. She was treated with 7 and 3 standard induction but retained significant residual disease on D14 marrow examination. Several salvage regiments were subsequently used without achieving remission. Her post therapy bone marrow biopsy still showed 20–30% leukemic blasts. Cells used in this study were extracted from post salvage marrow and analyzed using flow cytometry, revealing 46% of AML blasts to be CD19-postive/CD33-positive (Fig. 2A). To evaluate the anti-tumor effects of CD19b-CAR T-cells against the patient's CD19 + /CD33 + AML cells *in vitro*, AML cells were pre-stained with CellTracker CMTMR dye to distinguish target AML cells from effector T-cells. Co-culture assays were performed at an effector to target (E: T) ratio of 2:1 for 6 h, and flow cytometry analysis was conducted to analyze cell lysis activity caused by CD19CAR *versus* control T-cells (Fig. 2B).

After co-culture assay, CMTMR stained AML-1 patient cells were gated by CD19 and CD33, with the CD19 + /CD33 + population boxed in red. Compared to control T-cells, CD19b-CAR T-cells were able to eliminate 97.1% more CD19-positive/CD33-positive cells after 6 h of incubation. These results suggest that CD19b-CAR T-cells are robust in their anti-tumor properties (Fig. 2B).

At 6 and 24 h co-cultures with 2:1 and 5:1 effector T-cell to target ratios, CD19b-CAR- T-cells were able to lyse CD19 + /CD33 + cells of patient origin. CD19b-CAR T-cells were very potent, depleting tumor cells at 6 h co-culture. The results from E: T ratio of 2:1 and 5:1 were virtually the same, indicating that at ratios of 2:1, killing was saturated. CD19b-CAR T-cells were able to lyse over 90% of CD19 + /CD33 + cells when compared to control T-cells, showing that CD19b-CAR T-cells exhibit specific and potent target cell lysis ability (Fig. 2C).

4. Discussion

To our best knowledge, this is the first report demonstrating the effective cell killing and the potential application of CD19CAR T-cell therapy for CD19 + AML. CD19CAR could represent a powerful cellular therapy capable of eliminating target CD19 + AML cells as seen in our co-culture results. We found that even at a low effector: target cell ratio of 2:1, CD19CAR T-cells were able to effectively eliminate AML leukemia cells expressing CD19 in a 6 h co-culture.

We report here on the incidence of CD19 expression amongst 527 AML cases treated at a single institution. We show that AML, M2 was associated with the highest expression followed by AML, M4/5; AML, M0/1; and AML, NOS. CD19 was not expressed in AML, M3 (APL). Generally, CD19 expression in AML varies between 5% and 34% [9]. Mixed-phenotype acute leukemia had frequent expression of CD19, which was consistent with literature. Currently, there is no consensus on a standard treatment method for mixed-phenotype acute leukemia, and adult patient outcomes are poor when compared to other forms of leukemia, such as AML or ALL.

The use of CD19CAR as a standalone treatment would be most applicable for patients expressing CD19 in the vast majority of their leukemic cells, such as some cases of mixed-phenotype acute leukemia where CD19 can be expressed in up to 90–100% of AML blasts [10,11]. CD19CAR also has the potential to be combined with other treatments as a bridge to bone marrow transplant or to qualify patients for a more definitive therapy by reducing the tumor burden. Our results also suggest CD19CAR may have a promising role in treating or reducing

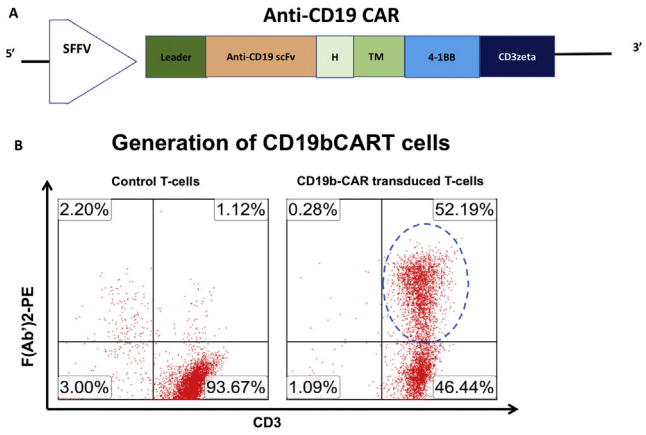

Fig. 1. Generation and expression of CD19b-CAR. (A) Schematic representation of the CD19CAR (B) Flow cytometry analysis of CD19b-CAR expression on the T-cell surface after transduction.

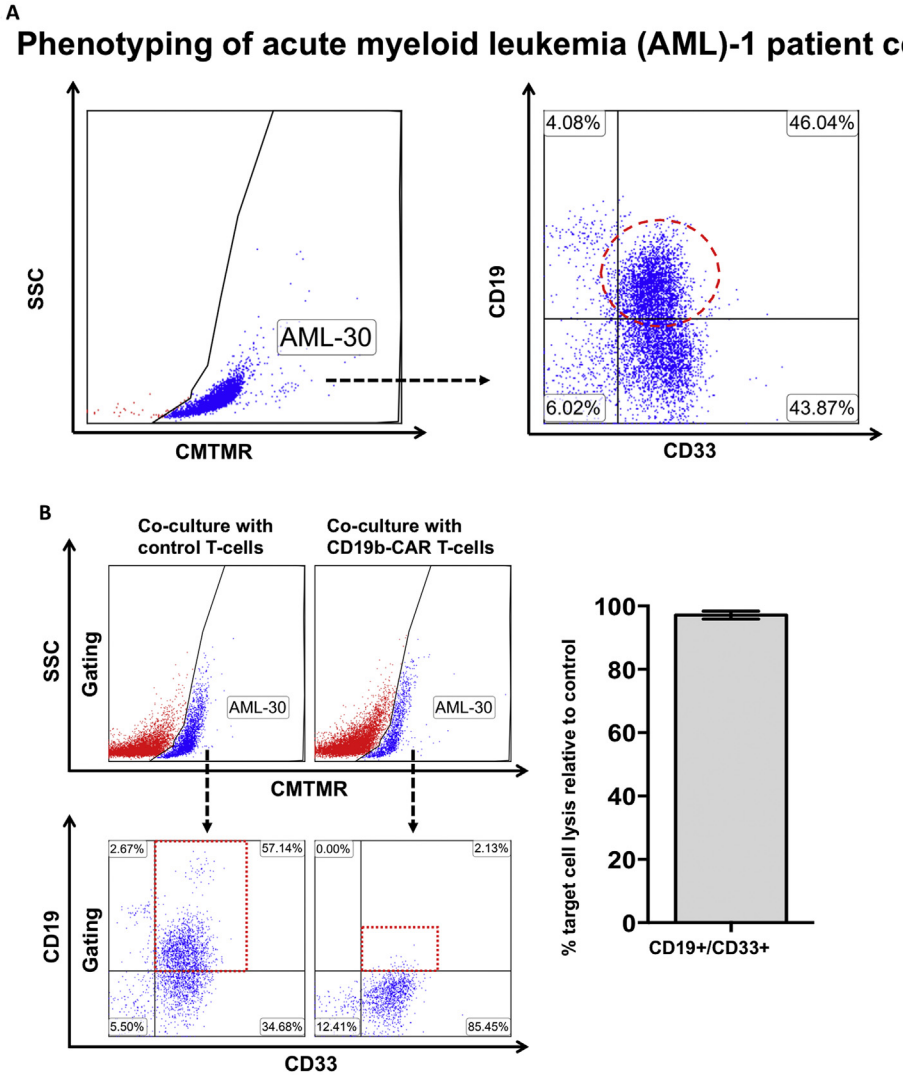

Fig. 2. CD19b-CAR- T-cells target and lyse primary CD19 + /CD33 + cells obtained from acute myeloid leukemia (AML) patient blood samples. (A) Flow cytometry was utilized to evaluate the population of CD19-postive/CD33-positive cells. (B) 6 h co-culture involving CD19CAR T-cells and primary CD19 + / CD33 + cells obtained from acute myeloid leukemia (AML) patient blood samples at an effector T-cell:target patient cell ratio of 2:1. (C) Bar graph displaying percent lysis results for CD19-positive/CD33-positive populations compared with CD19bCAR T-cells and control T-cells at effector T-cell: target patient cell ratio of 2:1 or 5:1 and 6 or 24 h co-culture.

tumor burden in patients with mixed-phenotype acute leukemia and CD19 + AML.

Conflict of interest

None.

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