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616. ACUTE MYELOID LEUKEMIAS: INVESTIGATIONAL DRUG AND CELLULAR THERAPIES

Fratricide-resistant anti-CD7 CAR-T cells for relapsed/refractory Acute Myeloid Leukemia

Marco Becilli[1], *Chiara Rosignoli*[1], *Francesca Del Bufalo*[1], *Pietro Merli*[1], *Mattia Algeri*[1], *Daria Pagliara*[1], *Francesco Quagliarella*[1], *Federica Galaverna*[1], *Valentina Bertaina*[1], *Maria Giuseppina Cefalo*[1], *Clelia Geraci*[1], *Giovanna Del Principe*[2], *Biagio De Angelis*[1], *Chiara Agrati*[1], *Concetta Quintarelli*[1], *Soheil Meshinchi*[3], *Dario Campana*[4], *Franco Locatelli*[1,5]

[1] IRCCS Bambino Gesù Children's Hospital, Department of Pediatric Hematology/Oncology and Cell and Gene Therapy, Rome, Italy
[2] IRCCS Bambino Gesù Children's Hospital, Transfusion Unit, Department of Laboratories, Rome, Italy
[3] Fred Hutchinson Cancer Research Center, Clinical Research Division, Seattle, United States
[4] Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore
[5] Catholic University of the Sacred Heart, Rome, Italy

Abstract Background Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only potentially curative treatment for patients with relapsed/refractory (r/r) acute myeloid leukemia (AML), but pre-transplant complete remission (CR) is required to minimize the risk of relapse post-transplant. Patients who fail to achieve CR or relapse early after allo-HSCT have a dismal prognosis, highlighting the urgent need for novel therapeutic options. Autologous T cells simultaneously expressing a 41BB-based chimeric antigen receptor (CAR) directed against CD7 and an anti-CD7 protein expression blocker (PEBL) to prevent fratricide, have been recently shown to be safe and effective in r/r T-cell acute lymphoblastic leukemia (T-ALL) (Oh BLZ et al, Nat Med. 2024). We hypothesized that this technology could be extended to r/r AML, where CD7 is variably expressed in approximately 15-30% of cases.
Methods At Ospedale Pediatrico Bambino Gesù of Rome, we infused autologous anti-CD7 PEBL CAR T cells (PCART7) in two patients with r/r CD7-positive AML under Hospital Exemption. Pt#1 is a 19-year-old male with t(10;17)(p15;q21) AML, relapsed 13 months after initial diagnosis, who failed second-line therapy including FLA, gemtuzumab-ozogamicin, Venetoclax, and progressed after bridging therapy with 5-Azacytidine/Venetoclax. Pt#2 is a 9-year-old male with t(7;19)(q22;q13) AML relapsed 2.6 years after allo-HSCT performed in CR2.
The PCART7 products were manufactured in our academic GMP-facility from cryopreserved leukaphereses using a lentiviral vector delivering anti-CD7 CAR and PEBL, supplied by MediSix Therapeutics®. T-cell engineering was performed during 12-day cultures in G-Rex chambers after $CD4^+$/$CD8^+$-cell enrichment. CAR-T cells were cryopreserved and tested prior to infusion. Both patients received a single infusion of PCART7 ($1x10^6$ CAR-T cells/kg) following cyclophosphamide/fludarabine-based lymphodepletion. Prior to lymphodepletion, bone marrow (BM) infiltration of leukemia blasts by multiparametric flow-cytometry (MFC) was 85% in pt#1 and 8.5% in pt#2. No extra-medullary localizations were evident.
Results PCART7 manufacturing yielded $1.0x10^9$ and $2.4x10^9$ total CAR-T cells. Cell viability was 80.2% and 89.7%, with 89.7% and 93.6% of cells expressing the CAR; vector copy number was 3.2 gc/cell and 4 gc/cell, respectively. One week after infusion, there was a remarkable expansion of PCART7 cells, peaking to 690.93/CAR^+ cells/mL in peripheral blood on day+14 in pt #1 and 236.63 CAR^+ cells/mL on day+12 in pt#2. In parallel, there was a rapid clearance of the $CD7^+$ cells which became undetectable by day+9 in both patients. CD7-negative T and NK cells recovered over time, retaining antiviral response. CAR^+ cells were detected in BM on the first assessment on day+14 (58.6% in pt#1, 31.2% in pt#2) up to the last evaluation before allograft (11.6% on day+50 and 19.8% on day +63, respectively). Grade 1 cytokine release syndrome (ASTCT consensus grading system) occurred in both patients between days 2 and 13, with fever and increase of C-reactive protein (up to 22.88 mg/dl in pt#1 and 6.58 mg/dl in pt#2) and ferritin (86426 and 14456 ng/mL). Both patients experienced grade 4 early- and late-immune effector cell-associated hematotoxicity (EHA/EBMT consensus grading system). Notably, grade 4 neutropenia (NCI-CTCA v5.0) was already present before lymphodepletion and persisted up to allograft engraftment. Immune effector cell-associated neurotoxicity syndrome did not occur. Infections were all manageable and limited to a probable invasive

pulmonary aspergillosis in pt#1, HHV-6 reactivation and BK virus-associated hemorrhagic cystitis in pt#2, who had a second peak of 490.49 CAR^+ cells/mL (predominantly $CD8^+$ cells) on day 30 after HHV-6 and BK virus reactivation.
Both patients received allo-HSCT from a matched unrelated donor, while in CR with negative measurable residual disease (MRD) on days 54 and 66 after PCART7 infusion. As of June 30th 2025, both patients are alive in continuous CR with negative MRD by MFC, 13 and 4 months after PCART7 infusion.
Conclusion This is the first demonstration that PCART7 may represent a valuable and feasible option for patients with r/r AML and high CD7 expression, offering the potential to achieve MRD-negative CR. The safety profile of the treatment is favorable and both T and NK cells CD7 negative can contribute to protect patients against infections.

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