Abstract Submission
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Preview

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Title: **CXCR4-modified CD33.CAR-CIK with enhanced bone marrow homing in Acute Myeloid Leukemia**

Type:

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Topic: * 1.: Pre-clinical data

Title

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Short title: * CXCR4-modified CD33.CAR-CIK with enhanced bone marrow homing in AML

Abstract text

Background: * Chimeric Antigen Receptor (CAR) Cytokine-Induced Killer (CIK) cell therapy is an emerging treatment for acute myeloid leukemia (AML) although some implementations are required. Specifically, it appears crucial to improve CAR-CIK infiltration ability into the bone marrow (BM) niche to eradicate leukemia stem cells (LSCs) at their location. CAR-CIK *ex vivo* manipulation influences the expression of several chemokine receptors and may dampen the capacity of infused cells to migrate to the BM. The chemokine ligand 12 (CXCL12), produced by mesenchymal stromal cells (MSCs) within the niche, and its chemokine receptor 4 (CXCR4) modulate leukocytes trafficking to the BM. In AML, CXCL12 binds CXCR4 overexpressed on blasts, enhancing their homing in the niche. CXCR4 expression is consistently downregulated during the culture of CIKs. Therefore, combining the expression of CD33.CAR and CXCR4 might promote CAR-CIK homing to the BM and subsequent leukemia eradication, specifically of LSCs.

Methods: * Two bicistronic Sleeping Beauty transposon vectors were designed: CXCR4(IRES)CD33.CAR and CD33.CAR(2A)CXCR4. The monocistronic CD33.CAR was used as control. Phenotype and CAR-related *in vitro* effector functions were compared. Migration *in vitro* toward rhCXCL12 or MSC supernatants was tested using a transwell migration assay. CAR-CIKs *in vivo* BM homing ability was evaluated in sub-lethally irradiated NSG mice. CAR-CIKs engraftment was assessed in BM, blood, spleen, and lungs

Results: * We noticed that both CD33.CAR(2A)CXCR4-CIKs (n=22, P<0.0001) and CXCR4(IRES)CD33.CAR-CIKs (n=9, P<0.0001) maintained CXCR4 overexpression during culture, whereas in CD33.CAR-CIKs was drastically downregulated (n=22). Nevertheless, CD33.CAR expression was lower in CXCR4(IRES)CD33.CAR-CIKs (n=8, P<0.0001) compared to CD33.CAR-CIKs, while CD33.CAR(2A)CXCR4-CIKs (n=11) exhibited a significant co-expression of both proteins against control (P=0.001). Therefore, CD33.CAR(2A)CXCR4 construct was chosen to proceed with further investigations. The typical CIK phenotype and memory subset composition was minimally affected by CXCR4 overexpression. Furthermore, CD33.CAR-CXCR4-CIKs maintained all CAR-related *in vitro* effector functions, eliminating $CD33^+$ KG1 and MOLM14 target cell lines, releasing cytokines (IL-2 and IFN-γ) and proliferating in an antigen-specific fashion. To determine the contribution of CXCR4-overexpression on CD33.CAR-CIK migration, CAR^+ CIKs were selected by immunomagnetic sorting. Notably, $CD33.CAR^+$-CXCR4–CIKs displayed improved migratory response toward rhCXCL12 as compared to CD33.CAR-CIKs (n = 10, P < 0.0001). Moreover, to mimic the conditions of the BM microenvironment, we employed supernatant of MSCs derived from healthy donors (HD) (n = 6) or AML patients at diagnosis (n = 10) as a chemotactic stimulus. CD33.CAR-CXCR4-CIKs still demonstrated an increased chemotactic response compared to control, which could be inhibited by the use of CXCR4 antagonist Plerixafor. Notably, 7 days after infusion into sub-lethally irradiated NSG mice, $CD33.CAR^+$-CXCR4-CIKs presented a significant increase in their ability to enter BM compartment when compared to $CD33.CAR^+$-CIKs, as demonstrated by the higher proportion of $hCD45^+$ cells retrieved in the BM. Contrarily, $CD33.CAR^+$-CXCR4-CIK frequency in peripheral blood and spleen displayed only minimal differences compared to control $CD33.CAR^+$-CIKs.

Conclusions: * Our data demonstrate CD33.CAR(2A)CXCR4-CIKs display enhanced *in vitro* migration and superior *in vivo* BM homing ability while maintaining CAR functionalities. This boosted migratory potential might facilitate an improved clearing of $CD33^+$ AML blasts and LSCs residing in the BM, which is currently being explored in ongoing experiments.

Clinical Trial Registry:

Disclosures: *	Dr Dotti is a paid consultant for Bellicum Pharmaceuticals, Tessa Therapeutics and Catamaran. The authors declare no conflict of interest. This research was funded by AIRC-5x10002018-21147 and PRIN 2017-NAZ-0412.
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