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Overexpression of CXCR4 Enhances the Efficacy of CAR-T Therapy for Acute Myeloid Leukemia

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Abstract

Chimeric Antigen Receptor (CAR) Cytokine-Induced Killer (CIK) cell therapy is a promising treatment for Acute Myeloid Leukemia (AML). However, it is hampered by dampened CAR-CIK cells infiltration in the bone marrow (BM) niche, where leukemia stem cells reside. Remarkably, CAR-CIK cells *ex vivo* manipulation affects the expression of several chemokine receptors, specifically CXCR4, and may abate engineered cells responsiveness to CXCL12 gradient, released by mesenchymal stromal cells (MSCs) within the niche.

Combining the expression of CD33.CAR and CXCR4 may improve CAR-CIK cells migration and retention within the BM and subsequent AML eradication. We designed two different bicistronic Sleeping Beauty transposon vectors to vehiculate the concomitant expression of CD33.CAR and $CXCR4^{WT}$ or the gain-of-function variant of CXCR4 (R334X), described in WHIM syndrome and responsible for leukocytes sequestration in the BM.

Overexpression of both $CXCR4^{WT}$ and $CXCR4^{MUT}$ was maintained during culture on CD33.CAR-CIK cells transduced with bicistronic vectors, whereas it was consistently downregulated on the monocistronic control. CD33.CAR was comparably expressed among the three different constructs. CD33.CAR, CD33.CAR-$CXCR4^{WT}$ or CD33.CAR-$CXCR4^{MUT}$-CIK cells maintained their phenotypic markers and memory phenotype. We then evaluated CAR-CIK cells

$CXCR4^{WT}$-CIK cells ($p < 0.0001$) and $CD33.CAR^{+}$-$CXCR4^{MUT}$-CIK cells ($p = 0.0015$) showed improved chemotaxis toward human recombinant CXCL12 compared to CD33.CAR-CIK cells (n = 10). Next, to better mimic the BM microenvironment features, we employed the supernatant of MSCs derived from healthy donors (HDs) or AML patients as a chemotactic stimulus and showed that both CXCR4-overexpressing CD33.CAR-CIK cells demonstrated increased chemotaxis toward HD- and AML-derived MSC supernatants compared to control (n = 14), which was abrogated by CXCR4 antagonist plerixafor.

To evaluate if CXCR4 overexpression may have an impact on CAR-associated effector functions, we tested $CD33.CAR^{+}$-, $CD33.CAR^{+}$-$CXCR4^{WT}$ and $CD33.CAR^{+}$-$CXCR4^{MUT}$-CIK cells against $CD33^{+}$ KG-1 AML cells. Similar cytotoxic activity, proliferative response and IFN-γ or IL-2 secretion levels were observed (n = 9) and even in the presence of CXCL12 their activation was not altered.

To assess if CXCR4-overexpressing CD33.CAR-CIK cells acquire improved BM homing *in vivo*, we infused NSG mice with either $CD33.CAR^{+}$-$CXCR4^{WT}$-, $CD33.CAR^{+}$-$CXCR4^{MUT}$- or $CD33.CAR^{+}$-CIK cells. The frequency and absolute number of $hCD45^{+}$ cells retrieved from BM, peripheral blood, and spleen was determined by FACS analysis 7, 10 or 14 days after transplant. Notably, CXCR4-overexpressing $CD33.CAR^{+}$-CIK cells displayed enhanced BM homing *in vivo*, linked with a prolonged retention of $CXCR4^{MUT}$.

To verify if CXCR4-overexpressing CD33.CAR-CIK cells achieve enhanced antitumor activity, we established a leukemia xenograft model with $CD33^{+}$ KG-1 AML cells. Four weeks after therapeutic cells infusion, animals treated with CXCR4-overexpressing CD33.CAR-CIK cells displayed a higher reduction of the frequency and absolute number of $hCD33^{+}$ cells in the BM compared to those receiving $CD33.CAR^{+}$-CIK cells (n = 12 mice per group). Moreover, CD33.CAR-CIK co-expressing $CXCR4^{WT}$ exerted a superior control of AML progression, with the median survival time increased from 57.5, 77.5, and 87.5 days in the untreated, $CD33.CAR^{+}$-CIK and $CD33.CAR^{+}$-$CXCR4^{MUT}$-CIK groups, respectively, to 110 days in the $CD33.CAR^{+}$-$CXCR4^{WT}$-CIK group ($p < 0.0001$, log-rank test).

Taken together, these data show arming CAR-CIK cells with CXCR4 may represent a promising strategy to increase their therapeutic potential for AML.

Disclosures

Dotti:*Bellicum Pharmaceuticals:* Consultancy; *Catamaran:* Consultancy. **Biondi:***Amgen:*

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Author notes

* Asterisk with author names denotes non-ASH members.

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