Abstract Submission 3rd European CAR T-cell Meeting

Preview

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Title: **Generation of CIK cells co-expressing CXCR4 and CD33.CAR with improved homing and antitumor activity for AML**

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Topic: * 3: Acute myeloid leukemia - Biology & Translational Research

Title

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Abstract text

Background: * Chimeric antigen receptor (CAR) T-cell therapy for acute myeloid leukemia (AML) has been quite elusive due to tumor heterogeneity, lack of ideal target antigens and the role of the tumor microenvironment in leukemia protection and leukemia stem cells (LSCs) maintenance. Therefore, CAR design must be improved for an effective anti-AML activity. Besides selecting a good target antigen, it is crucial to improve CAR T-cells infiltration, to eradicate LSCs at their location within the bone marrow (BM) niche. The chemokine receptor 4 (CXCR4) and its ligand, CXC motif ligand 12 (CXCL12) are two fundamental factors orchestrating leukocytes trafficking to the niche. In AML, the CXCL12-CXCR4 axis is co-opted by leukemic cells. CXCL12, released by mesenchymal stromal cells (MSCs), interacts with CXCR4 overexpressed on blasts, boosting their homing in the BM, where they can find a protecting environment. We hypothesize that CXCR4 overexpression by Cytokine-Induced Killer cells (CIKs) combined with a CD33.CAR may improve their homing to the BM and subsequent leukemia eradication.

Methods: * We designed two bicistronic vectors: CXCR4(IRES)CD33.CAR and CD33.CAR(2A)CXCR4. CIKs were non-virally engineered with the Sleeping Beauty transposon system to express our constructs or the monocistronic CD33.CAR, used as control. We assessed the chemotactic activity of the bicistronic constructs employing the transwell migration assay, toward rhCXCL12 and human MSCs supernatants. Additionally, *in vitro* effector functions were compared.

Results: * Considering that CXCR4 expression on CAR-CIKs is downregulated over culture, we induced it artificially. CXCR4(IRES)CD33.CAR-CIKs (n=8) expressed higher levels of CXCR4, but lower CD33.CAR expression compared with CD33.CAR-CIKs, while CD33.CAR(2A)CXCR4-CIKs (n=9) showed a significant co-expression of both proteins, as compared to control (P≤ 0.05). The analysis of T-cell markers and memory phenotype uncovered that CXCR4(IRES)CD33.CAR-CIKs (n=7) are enriched in $CD8^+$ compartment and include a higher fraction of T_{EMRA} contrary to CD33-CAR CIKs (P≤0.05), while CD33.CAR(2A)CXCR4-CIKs (n=8) phenotype is comparable to control. Chemotaxis assays toward rhCXCL12 confirmed that CXCR4(IRES)CD33.CAR-CIKs (n=7, P≤0.05) and CD33.CAR(2A)CXCR4-CIKs (n=7, P≤0.05) achieved a migration advantage over CD33.CAR-CIKs alone (n=11), with a mean percentage of migration of 58.5% and 68.8% respectively, compared to 40.5%. We were also able to observe a specific chemotactic response toward MSCs supernatants, as proved by the use of Plerixafor. Moreover, correlation analysis strengthened our observation, as higher CXCR4 expression resulted in increased migratory activity. Concerning CAR-related effector functions, CXCR4(IRES)CD33.CAR-CIKs and CD33.CAR(2A)CXCR4-CIKs displayed similar killing of the $CD33^+$ KG1 cell line, compared to CD33.CAR-CIKs alone, with a mean lysis of 56.6% (E:T ratio 5:1, n=7) for CXCR4(IRES)CD33.CAR-CIKs, and of 66.9% (n=9) for CD33.CAR(2A)CXCR4-CIKs, compared to a mean lysis of 62.5% (n=12) of CD33.CAR-CIKs. The bicistronic constructs also maintained their capacity to produce IL-2 and IFN-γ, and to proliferate after CD33 antigen exposure. CXCR4(IRES)CD33.CAR-CIKs displayed not significant but inferior effector responses as compared to control, due to lower CAR expression; CD33.CAR(2A)CXCR4-CIKs performed similarly to CD33.CAR CIKs alone.

Conclusions: * Compared to conventional CD33.CAR-CIKs, CXCR4-overexpressing CD33.CAR-CIKs exhibit enhanced migration capacity while retaining functional activity against $CD33^+$ target cells. Considering these results, we believe that CD33.CAR(2A)CXCR4 is the best candidate for further *in vivo* homing and anti-leukemic tests.

Clinical Trial Registry:

Disclosure: * Dr Dotti holds patents in the field of T cell engineering and has sponsor research agreements with Bluebird Bio and Bellicum Pharmaceutical. Dr. Dotti serves in the scientific advisory board of Bellicum Pharmaceutical and Catamaran. The authors declare no conflict of interest. This research

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