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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

CCR1-Targeting CAR T Cells for Acute Myeloid Leukemia

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BACKGROUND
Chimeric antigen receptor (CAR) T cell therapy is highly effective in patients with B cell malignancies and multiple myeloma, but initial clinical trials with CARs targeting acute myeloid leukemia (AML) have shown less successful results. Challenges inherent to using CAR T cells for AML include lack of a leukemia-specific target antigen due to heterogeneous antigen expression and potential on-target/off-tumor toxicity due to co-expression of target antigens on normal myeloid cells. C-C chemokine receptor 1 (CCR1, also known as CD191), a G protein-coupled receptor that binds to members of the C-C chemokine family, is a promising AML-associated antigen that is reported to be expressed on over 75% of AML samples and minimally expressed on hematopoietic stem/progenitor cells and T cells. The present study aimed to explore the potential of CCR1 as a novel CAR target antigen using a syngeneic mouse model that allows investigation of both efficacy and toxicity of the CAR T cells.
METHODS AND RESULTS
We developed a CAR based on the single chain variable fragment (scFv) KM5907 targeting both mouse and human CCR1. We constructed a second-generation CCR1-CAR containing a CD28 hinge, a CD28 transmembrane domain, a CD3 zeta signaling domain, and a CD28 co-stimulatory domain. We mutated the immunoreceptor tyrosine-based activation motifs (ITAMs) of the CD3 zeta chain to retain only a single active membrane-proximal (1XX) ITAM, based on a prior study showing that the 1XX ITAM format enhanced CAR T cell activity by reducing T cell exhaustion.
Primary murine T cells were retrovirally transduced and CCR1-CAR expression was assessed by flow cytometry. CCR1-CAR T cells promoted effective elimination of BM185 leukemia cells overexpressing murine CCR1 *in vitro*. Similarly, primary human T cells transduced with the CCR1-CAR exhibited potent cytotoxicity against C1498 leukemia cells overexpressing human CCR1 *in vitro*.
To evaluate anti-leukemia activity of the CCR1-CAR T cells *in vivo*, BALB/c mice were sublethally irradiated and engrafted with $1x10^5$ BM185 leukemia cells modified to overexpress murine CCR1. Two days later, mice were treated with $2x10^6$ CCR1-CAR T cells and monitored for weight changes and survival. Growth of BM185 leukemia cells (expressing firefly luciferase) and expansion of CAR T cells (expressing gaussia luciferase) were assessed via two-color bioluminescence imaging (BLI). On day 6, mice were bled retro-orbitally and serum was analyzed for cytokine levels. The CCR1-CAR T cells demonstrated potent anti-leukemia activity and T cell expansion *in vivo*, promoting improved overall survival compared with control mice that received only tumor cells (Figure 1). CCR1-CAR T cell activity was accompanied by mild, self-limited toxicity, characterized by transient weight loss (Figure 2), but not significant elevation of serum levels of cytokines associated with cytokine release syndrome (CRS) such as IL-6 and TNF-α. BLI of the CCR1-CAR T cells revealed enhanced trafficking into the bone marrow and spleen followed by proliferation in irradiated mice even in the absence of tumor, suggesting that CCR1-CAR T cells are stimulated by CCR1 antigen expressed on non-leukemic host cells in addition to leukemia cells.
CONCLUSIONS
In summary, CCR1-CAR T cells represent a promising therapeutic strategy for AML that to our knowledge has not yet been evaluated in preclinical or clinical studies. We generated CCR1-CAR T cells that demonstrated potent anti-leukemia activity *in vitro* and *in vivo* in a syngeneic mouse model. Clinical signs of self-limited toxicity were not associated with elevated levels of inflammatory cytokines and might be caused by an on-target/off-tumor effect, which warrants further investigations to better characterize the safety profile of CCR1-CAR T cells. The CCR1-CAR T cells also displayed potent cytotoxicity *in vitro* via

human CCR1, which will facilitate clinical translation of the findings. A CCR1-CAR may also offer benefit in other hematologic malignancies with reported CCR1 expression such as multiple myeloma or Hodgkin's lymphoma.

Disclosures James: *MSKCC:* Patents & Royalties: Pending patents related to leucine zipper sorting technology. **van den Brink:** *Thymofox:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Da Volterra:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *GlaxoSmithKline:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Vor Biopharma:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Wolters Kluwer:* Patents & Royalties; *Juno Therapeutics:* Other: IP licensing; *DKMS (a non-profit organization):* Membership on an entity's Board of Directors or advisory committees; *Lygenesis:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Pluto Immunotherapeutics:* Consultancy, Current holder of *stock options* in a privately-held company, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Ceramedix:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Notch Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Nektar Therapeutics:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Frazier Healthcare Partners:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Rheos Medicines:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees; *Seres Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company, Honoraria, Membership on an entity's Board of Directors or advisory committees, Other: IP licensing , Research Funding.

Figure 1

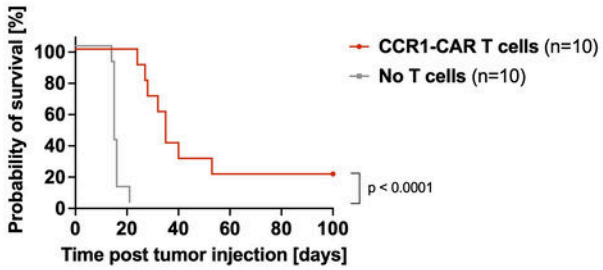

Figure 1: Survival of BALB/c mice inoculated with CCR1-overexpressing BM185 leukemia cells with or without CCR1-CAR T cell treatment. Data were pooled from two independent experiments.

Figure 2

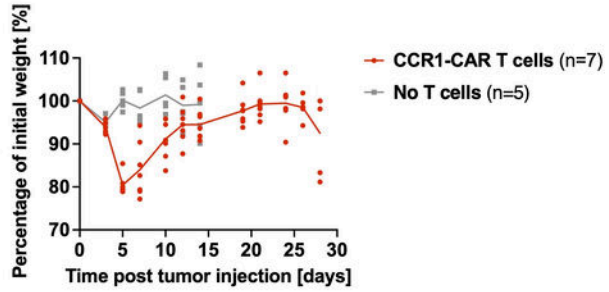

Figure 2: Serial weight assessments of BALB/c mice inoculated with CCR1-overexpressing BM185 leukemia cells with or without CCR1-CAR T cell treatment. Data from one representative experiment are shown.

Figure 1

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