FULLY HUMAN CD123 CAR T CELLS IRRADICATE AML IN PRE-CLINICAL MODELS AND EXHIBIT A FAVORABLE SAFETY PROFILE

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Background CD123, the interleukin-3 receptor alpha chain (IL-3Ra), is widely overexpressed in hematologic malignancies including acute myeloid leukemia (AML), at both the level of leukemic stem cells (LSCs) and leukemic blasts. This makes CD123 an attractive therapeutic target. Monoclonal antibodies directed against CD123, and CD123 chimeric antigen receptor (CAR)-modified T cells are explored for new immunotherapies for the treatment of AML. Here, we developed a second generation anti-CD123 CAR comprising a fully human CD123 targeting scFv domain, a 4-1BB (CD137) co-stimulatory domain, and CD3ζ activation domain, and investigated its potency against AML.

Methods Primary human T cells from healthy donors were enriched and transduced with lentiviral vector encoding the CD123 CAR, and CAR expression was detected by flow cytometry. CAR123 T cells or untransduced UTD control T cells co-cultured with the CD123+ MOLM-14 and KG1a AML cells, or the CD123-293T cells for 18h, were evaluated by luciferase assay, and cytokine production was measured by ELISA. Supernatants were collected from co-cultures of CAR T cells with CD123+ MOLM-14 target cells, and the concentration of cytokines IL-2, IFN-γ, and TNF-α was determined. CAR123 T cell functionality in vivo was evaluated in a MOLM-14 acute myeloid leukemia NSG xenograft model exhibiting CD123+ phenotype. In addition, myelotoxicity was investigated in colony-forming unit assays.

Results Lentiviral transduction resulted in robust expression of CD123 CAR in the transduced T cells. The CD123 CAR T cells showed potent in vitro killing of CD123+ AML cell lines MOLM-14 and KG1a, but no killing of the CD123-293T cells, indicating CD123-dependent efficacy. The TNF-α was modestly elevated in culture supernatants from CD123 CAR T cells co-incubated with target cells, however there was no significant induction of IL-2 or IFN-γ, suggesting low risk of cytokine release syndrome. Colony-forming unit assays utilizing peripheral blood CD34+ hematopoietic stem cells from healthy donors treated with CAR123 T cells overnight, yielded similar numbers of BFU-E erythroid and CFU-GM myeloid colonies to an untransduced T cell control, indicating an absence of CAR123-associated myelotoxicity. Moreover, CAR123 T cells showed efficient tumor clearance, expansion and persistence in vivo, and no apparent toxicity.

Conclusions In summary, the fully human CAR123 T cells are highly potent against AML in vitro and in vivo, manifest the desired safety attributes, and are a promising modality for AML therapy.