

DEVELOPMENT OF ANTI-MUCIN1 CAR-T AGAINST SOLID TUMORS

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Background Chimeric antigen receptor (CAR) -T cell therapies have proven to be effective against various liquid tumors. However, the development of CAR-T against solid tumors has been challenging due to insufficient efficacy and potential on-target off-tumor toxicities caused by low expression of tumor antigens on normal tissues. Testing various affinities of CARs has demonstrated that lower affinity CARs maintain its anti-tumor effect while minimizing safety concerns (1). In order to develop a CAR-T against solid tumors expressing Mucin1, we have screened for Mucin1 binding antibodies and tested their anti-tumor effect in vitro and in vivo. The potential of on-target off-tumor toxicity was also measured in vitro.

Methods Anti-Mucin1 human single chain variable fragments (scFv) were obtained via screening against a scFv display library. Anti-Mucin1 scFvs were incorporated into CARs and in vitro, in vivo functions against various tumor cells expressing Mucin1 were tested. For in vivo studies, tumor bearing NOG mice (HCC1954 cells) received anti-Mucin1 CAR-T cells. Therapeutic efficacy was evaluated by measuring tumor volumes. Potential on-target off-tumor toxicity against Mucin1 on normal cells was tested by investigating the killing effect of anti-Mucin1 CAR-T against cancer cell line (HCC70) and non-tumorigenic breast epithelial cell line (MCF-10A) in co-culture systems

Results In vitro activity of anti-Mucin1 CAR-T cells that displayed a range of affinities for Mucin1 (27nM to 320nM) showed similar cytokine secretion levels and cytotoxicity against Mucin-1 expressing tumor cell lines (HCC70 and T47D). Robust anti-tumor activity was also demonstrated in vivo against large tumors (400~500 mm³) with relatively small numbers of CAR-T cells (0.5 x 10⁶ CAR-T cells per mouse). In vivo expansion of CAR-T cells were observed in all scFv-CAR-T cases and accompanied by close to complete regression of tumors within 25 days post CAR-T cell injection. Of the 4 scFv CAR-Ts, 2H08 (with a Kd of 94nM) was tested for activity against normal breast epithelial cells. When 2H08-CAR-T was cocultured with a mixture of HCC70 and MCF-10A cells, they preferentially killed only the Mucin1 overexpressing HCC70 cells leaving MCF-10 cells intact.

Conclusions Our study demonstrates anti-tumor activity of a novel scFv-derived CAR-T recognizing Mucin1 and its effectiveness in large pre-established tumors in vivo. We also demonstrate that 2H08-CAR-T can distinguish between target overexpressing cancer cells and normal epithelial cells, which suggests that by toning down the affinity of CAR against antigen one can improve the safety profile of solid tumor antigen targeting CAR-T cell therapies.

REFERENCE

1. Castellarin M, Sands C, Da T, Scholler J, Graham K, Buza E, Fraietta J, Zhao Y, June C. A rational mouse model to detect on-target, off-tumor CAR T cell toxicity. *JCI Insight* 2020; 5:e136012

Ethics Approval All experiments were done under protocols approved by the Institutional Animal Care and Use Committee (IACUC) (Study#LGME21-011).

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying

images. A copy of the written consent is available for review by the Editor of this journal.

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