213 AB-X INTEGRATED CIRCUIT T CELLS DEMONSTRATE IMPROVED POTENCY, EXPANSION, AND SPECIFICITY COMPARED TO MSLN CAR T CELLS

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Background In solid tumors, CAR T cell efficacy is limited by off-tumor toxicity and suppression by the tumor microenvironment (TME). AB-X is an integrated circuit T cell (ICT cell) intended for the treatment of ovarian cancer. AB-X includes a transgene cassette with two functional modules: 1) an "AND" logic gate designed to limit off-tumor toxicity through dual tumor antigen recognition; 2) a dual shRNA-miR to resist TME suppression and improve ICT cell function. The AB-X logic gate consists of a priming receptor that induces expression of an anti-mesothelin (MSLN) CAR upon engagement of a ALPG/P (alkaline phosphatase germ-line/placental). The dual shRNA-miR mediates downregulation of FAS and PTPN2. The AB-X DNA cassette is inserted into the T cell genome at a defined novel genomic site via CRISPR-based gene editing.

Methods Dual-antigen specificity of the logic gate was assessed in mice harboring MSLN+ and ALPG/P+MSLN+ K562 tumors established on contralateral flanks. Potency was measured in a subcutaneous MSTO xenograft model. Logic-gated ICT cells were compared with MSLN CAR T cells in both models. In vitro, expansion of ICT cells with the FAS/PTPN2 shRNA-miR was evaluated in a 14 day repetitive stimulation assay (RSA). In vivo, expansion and potency were measured in the MSTO xenograft model. An in vitro FAS cross-linking assay was conducted to assess the impact of FAS knockdown on FAS-mediated apoptosis.

Results Logic-gated ICT cells demonstrated specific activity against ALPG/P+MSLN+ tumors, but had no effect against MSLN+ tumors in the K562 in vivo specificity model. In addition, logic-gated ICT cells demonstrated greater in vivo potency than MSLN CAR T cells in the MSTO xenograft model. In our RSA, ICT cells containing the FAS/PTPN2 shRNA-miR had 8-fold greater expansion than the MSLN CAR T cells. Enhanced expansion was confirmed in vivo with ICT cells demonstrating >10-fold expansion in tumors and peripheral blood, enabling comparable growth inhibition in MSTO xenografts at less than one quarter the dose of the MSLN CAR T cells. Importantly, PTPN2 knockdown resulted in balanced expansion of all T cell subsets, including CD45RA+, CCR7+ memory cells. Lastly, ICT cells containing the FAS/PTPN2 shRNA-miR were resistant to FAS-mediated apoptosis.

Conclusions AB-X ICT cells specifically recognize ALPG/P+MSLN+ tumors, demonstrate superior potency, expansion, and persistence compared with MSLN CAR T cells, and are resistant to ovarian TME suppression. AB-X will be evaluated in clinical trials for treatment of platinum resistant/refractory ovarian cancer.

Acknowledgements We would like to acknowledge all of our colleagues at Arsenal Biosciences, without whom this work would not have been possible.

http://dx.doi.org/10.1136/jitc-2021-SITC2021.213