188 ARGININE PRE-CONDITIONING ENHANCES T-CELL POTENCY AND METABOLIC FITNESS

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Background Enhanced T-cell performance is imperative for the success of adoptive T-cell-based therapies. Here we assessed the killing efficiency of MART-1 specific TCR T-cells after Arg, Gln, and Leu pre-conditioning using the Agilent xCELLigence RTCA eSight (eSight). The eSight system can simultaneously capture live-cell images and measure cellular impedance, which determines the changes in cell number, morphology, and attachment. In parallel, the status of metabolism of the pre-conditioned T-cell was determined by Seahorse XF assays.

Methods Briefly, $CD3^+$ T-cells (Hemacare, Seattle, WA) were transduced with retrovirus SAMEN-DMF5 with a CD34 marker gene, against MART-1. The T-cells were pre-conditioned in a range of concentrations varying between 0–6mM with Arg, Glu and Leu for 7 days, followed by a killing assay using MART-1 expressing melanoma cell line as target cells (624.38) engineered to express a red-fluorescent nuclear protein, and Seahorse XF Cell MitoStress and T-cell metabolic profiling assays. The comparison was made with the controls, including transduced T-cells grown in RPMI but with no amino acids during pre-conditioning (TCR_RPMI), and non-transduced T-cells (Non_TCR).

Results Our data show Arg supplementation enhanced T-cell killing of target cells while Leu and Gln suppressed T-cell mediated killing. Specific cytolysis (5:1, E:T ratio) at 60h for TCR_RPMI and 6 mM Arg pre-conditioned T cells (TCR_ARG) were 35% and 76%, respectively. We further determined the impact of the duration of pre-conditioning. 2 and 4 days of pre-conditioning with 6 mM Arg were done along with the 7-day method. The percentages of cytolysis of TCR_ARG (6 mM) at 60 hr were 29, 68, and 81 on days 2, 4, and 7, respectively, indicating that improved killing potency of the T-cells started as early as 4 days with Arg. The Seahorse XF assays were performed to determine metabolic fitness and persistence. Arg (6mM) supplementation increased the spare respiratory capacity 1.7 and 2.6 times compared to TCR_RPMI control on days 4 and 7 respectively.

Conclusions In summary, Arg at 6mM concentration was effective with the highest percentage of cytolysis and a higher spare respiratory capacity and Gln and Leu conditioning suppressed T-cell killing. Consistently, Arg fuels the TCA cycle and thus, shifts metabolism towards mitochondrial respiration.

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