CAR T CELLS UNDERGOING EPIGENETIC
REPROGRAMMING BY LOW-DOSE DECITABINE
ENHANCES PERSISTENT ANTI-TUMOR EFFICACY IN VIVO

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Background Anti-CD19-directed chimeric antigen receptor (CAR) T-cell therapy has had a resounding effect on the treatment of B-ALL. However, CAR T cells have been less effective against B-cell non-Hodgkin lymphoma (B-NHL), in part because they become a exhausted state triggered by chronic antigen stimulation and characterized by upregulation of inhibitory receptors and loss of effector function. 1-4 It has recently been demonstrated that de novo DNA methylation promoted T-cell exhaustion, whereas methylation inhibition enhanced ICB-mediated T-cell rejuvenation in vivo. 5, 6 FDA-approved DNA demethylating agents, such as decitabine (DAC), may provide a means to modify exhaustion-associated DNA methylation programs that restrict ICB-responsiveness.

Methods We treated CAR (CAR-CD19-expressing) T cells with low-dose DAC (dCAR T cells), to determine its effects on antitumor activities, exhaustion- and memory-associate cell phenotype change, cell cytokine production, and cell proliferation. Its impact on antitumor activities was evaluated in vitro functional assays and mouse in vivo studies. We also conducted western blot, flow cytometry, methylation analysis, RNA in situ hybridization and high throughput RNA sequencing to determine the underlying mechanisms of dCAR T cell function.

Results The low-dose, short-term DAC treatment in vitro enhanced the central memory (Tcm) population and the ration of CD4/CD8, and induced degradation of DNMT3. CAR T cell treated by DAC developing into less-differentiation status by enhancing memory. dCAR T cells exhibit enhanced antitumour reactivity and the maintenance of a memory-like phenotype at low effector:target ratios. Especially shown by the ‘stress test’, the dCAR T cells at very low doses could efficiently control tumours with a very large burden, and have effective recall responses upon tumour re-challenge in vivo. Importantly, the dCAR T cells maintained a higher proportion of cells with a memory phenotype than did the CAR T cells under long-term tumour stimulation. Transcription of gene sets involved in memory maintenance, proliferation, cytokine production and anti-inhibitor processes was triggered by antigen-expressing target cells upon DAC exposure before antigen stimulation. dCAR T cells avoided the exhaustion programme induced during tumour cell stimulation; they did not upregulate the expression of genes encoding inhibitory receptors and retained relatively high expression of memory related transcription factors and genes.

Conclusions CAR T cells underwent DNA reprogramming after DAC treatment, which induced significant sustained cell expansion, cytotoxicity, and cytokine production and reduced exhaustion after antigen exposure.

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**Abstract 771**

**CHARACTERIZATION OF TUMOR INFILTRATING IMMUNE CELLS FROM ADULT SOFT TISSUE SARCOMAS**

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**Background** Sarcoma is a group of rare bone and soft tissue tumors with over 50 distinct subtypes. Survival rate ranges widely due to the lack of efficacious treatments. Immunotherapy, such as adoptive cell therapy (ACT), has drawn great interest due to its minimal toxicity. In ACT, tumor infiltrating lymphocytes (TILs) are isolated from patients, expanded, and autologously reinfused back. We recently observed TILs’ presence in Undifferentiated Pleomorphic Sarcoma (UPS) and Myxofibrosarcoma (MFS) tumors and found that tumor’s PD-L1 overexpression is correlated with better clinical outcome in UPS but not MFS. The Thelper1 inflammatory pathway was highly activated in the former subtype, which may explain the better outcome. These results illustrate the immunological differences where TILs may play a critical role. We hypothesize that there are phenotypic and functional differences between TILs of UPS and MFS that may be related to clinical outcomes. Sarcoma TILs are rare and challenging to culture.

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**REFERENCE**


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