

Title: Decitabine Treatment of Tumor-Infiltrating Lymphocytes (TIL) During Ex Vivo Expansion Induces a More Memory-like Phenotype, Reduces Inhibitory Receptor Expression, and Increases Functionality

Background: Adoptive cell therapy using autologous tumor-infiltrating lymphocytes (TIL) has shown durable responses in a subset of patients with metastatic melanoma (Sarnaik AA et al, *J Clin Oncol* 2021), cervical carcinoma (Jazaeri AA et al, ASCO 2019 #182), and other epithelial malignancies. While TIL can be reactivated and expanded ex vivo, their epigenetic programming could be keeping TIL in a more differentiated and less functional state. De novo DNA methylation has been shown to promote and reinforce the development of T-cell exhaustion and represents a cell-intrinsic barrier to reinvigoration. The use of low-dose decitabine (DAC) treatment, a DNA hypomethylation agent, has been shown to confer some level of epigenetic reprogramming on exhausted T cells and has been shown to endow CAR T-cells with enhanced persistence, memory phenotype, and antitumor potential. In this study we investigated whether low-dose DAC treatment during ex vivo TIL expansion could improve the quality and function of TIL by reducing their effector differentiation and maintaining a population of more memory-like cells.

Methods: Patient tumors from different indications were received, fragmented, and placed in media with 6,000 IU/mL of IL-2 for 11 days for a pre-Rapid Expansion Protocol (pre-REP). Pre-REP TIL were then propagated in a REP protocol with irradiated PBMCs, anti-CD3 antibody, and 3,000 IU/ml of IL-2 for another 11 days. Different doses (10nM, 30nM, 100nM) of DAC were added to the culture at the initiation of either the pre-REP and REP stage or the REP stage only. The expansion potential as well as the phenotypic and functional characteristics of post-REP TIL were then evaluated.

Results: While DAC treatment led to a decrease in TIL expansion and an increase in the CD4⁺ to CD8⁺ ratio, this coincided with an increase in the frequency of central memory-like cells (CD45RA⁻CCR7⁺) as well as the expression of IL-7R and the transcription factors TCF1, Eomes, and KLF2, suggesting a shift towards a more memory-like phenotype. Additionally, DAC treatment increased the expression of CD25, CD28, and ICOS while reducing the expression of inhibitory receptors like PD-1 and TIGIT. Following stimulation, DAC-treated TIL showed increased degranulation and a higher frequency of IFN γ ⁺TNF α ⁺ cells, which translated into increased cytotoxicity.

Conclusions: DAC treatment during TIL expansion can shift the balance away from effector differentiation and towards a more memory-like phenotype, while conferring increased expression of co-stimulatory receptors, reduced expression of inhibitory markers and improved functionality. Inhibiting DNA methylation programs during TIL expansion could represent a useful approach for modifying the epigenetic landscape of TIL, imprinted prior to ex vivo expansion and introduced during the expansion process itself, to improve their therapeutic potential.