

## VITAMIN C TREATMENT PREVENTS CAR T CELL EXHAUSTION, MAINTAINS STEM CELL PHENOTYPE AND ENHANCES ANTITUMOR FUNCTION

Leonce Kouakanou\*, Hanmin Wang, Grace Sun, Tristan Chan, Jennifer Shepphird, Darya Alizadeh, Christine Brown, Leo Wang. *City of Hope, Duarte, CA, USA*

**Background** Chimeric antigen receptor (CAR) T cells have emerged as an effective therapy for B cell malignancies. However, major limitations to efficacy are lack of T cell persistence and T cell exhaustion, which result from epigenetic repression. Previous reports demonstrated that epigenetic remodeling can prevent T cell exhaustion and enhance CAR T antitumor activity.<sup>1,2,3</sup> Vitamin C (VC) was previously described to modulate immune cell function through epigenetic modification.<sup>4,5</sup>

**Methods** Here we sought to determine the effect of VC on CD19-CAR T cell functional differentiation. CD3<sup>+</sup> T cells were activated and transduced with lentiviral supernatant in the presence of IL-2, low IL-15, and VC. The effect of VC on T cell differentiation was determined using flow cytometry, and on proliferation by direct cell count. To analyze functional differences between control (without VC) and VC-manufactured CD19-CAR T cells (VC-CD19-CAR T), we used a repeat challenge assay in which CAR T cells were cocultured with tumor cells, with fresh tumor cells reintroduced each week for four weeks in total. CAR T cell expansion, differentiation, and exhaustion were determined using flow cytometry and cell count. Cytotoxicity was assessed by measuring lactate dehydrogenase release. Whole-genome bisulfite sequencing was performed to analyze cell-intrinsic changes in CAR T cell DNA methylation as a result of VC treatment. Xenograft tumor mouse models were used to evaluate the long-term *in vivo* function of VC-CD19-CAR T cells.

**Results** Our data showed that addition of VC during CD19-CAR T cell production enforces a stem cell memory-like phenotype, characterized by increased expression of CD45RA<sup>+</sup>CD62L<sup>+</sup> and CD27<sup>+</sup>CD62L<sup>+</sup>. VC-CD19-CAR T cells expanded more during manufacturing than controls, and their higher rate of expansion was preserved during the extended *in vitro* challenge. Interestingly, VC-CD19-CAR T cells were better at killing target cells in the first exposure to target cells, and retained their killing ability after subsequent stimulations while control CAR T cells did not. The improved function was mirrored by phenotypic analysis showing that stimulated VC-CD19-CAR T cells retained a stem cell memory-like phenotype with a lower expression of exhaustion markers after the last stimulation compared to control. *In vivo*, VC-CD19-CAR T cells efficiently delayed tumor growth resulting in superior overall survival compared with control.

**Conclusions** Our studies demonstrate that the addition of VC to CD19-CAR T production increased their expansion and prevented their exhaustion, resulting in enhanced antitumor function. Studies are ongoing to determine whether this occurs via epigenetic reprogramming.

### REFERENCES

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**Ethics Approval** Human samples were obtained from healthy donors under protocols approved by the City of Hope IRB (number 16025)

All mouse experiments were performed using protocols approved by the City of Hope IACUC (number 16081)

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0351>