

DIACYLGLYCEROL KINASE ALPHA AND ZETA DUAL INHIBITORS ENHANCE T CELL RESPONSES AND PROMOTE ROBUST AND DURABLE ANTI-TUMOR T CELL IMMUNITY

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Background Diacylglycerol kinases (DGK) convert diacylglycerol (DAG) into phosphatidic acid which limits DAG-induced propagation of T cell activation.¹ T cells predominantly express DGK alpha and DGK zeta isoforms (DGKalpha/zeta),² and deletion of either enhances T cell activation and function.^{3,4} DGKalpha/zeta are overexpressed in tumor infiltrating T cells and may limit the anti-tumor activity of these cells.⁵ Thus, inhibition of DGKalpha/zeta is a potential therapeutic avenue to enhance T cell anti-tumor responses. We demonstrate that inhibition of DGKalpha/zeta with novel dual DGKalpha/zeta inhibitors (DGKi) enhances anti-tumor T cell activity.

Methods The role of DGKalpha/zeta in T cell activation was studied using wildtype and CRISPR-knockout T cells. *In vitro* studies assessing the effect of DGKi were conducted using various mouse and human T cell functional assays. T cell exhaustion studies were performed in T cells from lymphocytic choriomeningitis virus (LCMV) clone 13-infected mice. *In vivo* activity of DGKi was assessed by measuring T cell activation in OT-I mice challenged with OVA-peptides. Efficacy studies and memory rechallenge experiments were performed in the MC38 syngeneic tumor model.

Results CRISPR knockout studies characterizing the role of DGKalpha/zeta on T cells demonstrated that both isoforms regulate T cell activation and function. DGKi enhanced IFN gamma production by human melanoma-specific T cells stimulated with the weak affinity MART1 antigen. Similarly, treatment of mouse OT-I cells with DGKi enhanced responses to low-affinity OVA-peptides and OT-I-mediated cytotoxicity of MC38-OVA tumor cells. Treatment of exhausted CD8 T cells with DGKi reverted exhaustion caused by chronic LCMV infection, and enhanced T cell function with PDL1 blockade. DGKi rescued cytokine release from T cells suppressed by adenosine, TGF beta or PGE₂ treatment. Oral administration of DGKi resulted in dose-dependent increase in T cell activation in mice. Furthermore, DGKi in combination with anti-PD-1 therapy was efficacious in the MC38 model with complete tumor regressions in treated animals. These mice eradicated tumors upon rechallenge with MC38 cells two months later, demonstrating durable anti-tumor memory responses.

Conclusions We demonstrate that DGKalpha/zeta negatively regulates T cell activity. Dual DGKalpha/zeta inhibition restores T cell responses to weak tumor antigens, overcomes cell exhaustion, and enhances CD8 T cell cytotoxic activity. *In vivo* efficacy data demonstrates that DGKi in combination with anti-PD-1 antibody therapy promotes robust anti-tumor responses and generates durable T cell memory. These data support dual DGKalpha/zeta inhibition in combination with anti-PD-1 antibodies as a therapeutic approach for cancer treatment.

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Ethics Approval All animal studies were approved and conducted in accordance with the Explora IACUC Program of Veterinary Care.

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