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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Mitochondrial Reprogramming By Bcl-2 Inhibitor Venetoclax Enhances αCD19 CAR-T Cell Fitness and Anti-Tumor Efficacy

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Introduction

Patients undergoing anti-CD19 chimeric antigen receptor T cell (CAR T) therapy for the treatment of aggressive B-cell non-Hodgkin lymphomas (B-NHL) can expect a complete response rate of merely 43-68%. Insubstantial clinical responses to CAR T therapy and a small window of survival for patients facing relapsed/refractory (R/R) disease highlights the critical need for improvement of CAR T therapy in aggressive B-NHL cases. Recent findings suggest that enriching for a $CD8^+$ $T_{Stem\ Cell\ Memory}$ (T_{SCM}) population within adoptive cell therapies translates to enhanced antitumor function and persistence *in vivo*, and thus produces favorable clinical outcomes. T_{SCM} cells display heightened self-renewal, low mitochondrial membrane potential ($\Delta\Psi m$), and are metabolically dependent upon oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO). Selection of $CD8^+$ T_{SCM} subsets is therefore necessary to ensure a robust antitumor response. Previously, we revealed synergy between the Bcl-2 inhibitor, Venetoclax, and CAR T cells in the context of B-NHL. Venetoclax exposure increased $CD8^+$ T_{SCM} cell frequency, decreased $\Delta\Psi m$, reduced $T_{Regulatory}$ (T_{REG}) cell frequency, and bolstered effector cytokine production. Though the role of Bcl-2 inhibition within B-NHLs is well characterized (FDA-approved for frontline and relapsed/refractory chronic lymphocytic leukemia [CLL] treatment), further investigation regarding the impact of Venetoclax on the patient T cell compartment is needed. In this study, we demonstrate a role for Venetoclax as a qualitative enhancer of bulk CAR T cell product using both healthy-donor and B-NHL patient-derived T cells, and implicate modulation of mitochondrial pathways as a likely mechanism of action.

Methods

Whole peripheral blood mononuclear cells were collected from consenting healthy human donors or CLL patients undergoing ramp-up with Venetoclax (BDR 164122) at Roswell Park Comprehensive Cancer Center, and activated with αCD3/αCD28. **CLL samples** were collected at baseline, 1 day, 7 days, and 1-month timepoints. **Transduction** was performed with lentiviral vectors encoding CD28 or 4-1BB-based α-CD19 CAR constructs 24 hours post-activation. Transduced cells were cultured in RPMI with hIL-2 and hIL-7. ***In vivo* studies** were performed on 7-week-old SCID mice using Raji-Luc, Rec-1, and U2932. Venetoclax was given daily [100mg/kg] via oral gavage. CAR T cells were delivered by single infusion via tail-vein injection at a dose of either $1x10^6$ or $3x10^6$ cells total.Mice were imaged via IVIS spectrum. **Western blot** was performed on stimulated untransduced T cells or CAR T cells exposed to Venetoclax. Total protein was quantified, and membranes were probed for apoptotic and mitochondrial protein expression. **Patient sample immunophenotyping** was performed on CLL patient samples using panels interrogating memory differentiation, exhaustion, and T_{REG} populations.

Results

Consistent with our hypothesis, a significant increase in mitochondrial proteins associated with FAO and OXPHOS - ACC, PDH, and AMPKα - was observed in exposed CAR T cells, as well increased expression of transcription factor FOXO1 - an essential driver of $CD8^+$ memory differentiation. Voltage-dependent anion channel (VDAC) expression increased, suggesting heightened efflux of respiratory substrates into the tricarboxylic acid cycle. Hexokinase-I, a critical glycolytic enzyme, was significantly downregulated. We next investigated our combination therapy *in vivo* and observed extended survival outcome by 15 days. Further *in vivo* studies investigating preclinical models of mantle cell lymphoma and activated B-cell diffuse large

B-cell lymphoma are currently ongoing. Within our CLL patient samples, 24-hour and 7-day timepoints reflected a favorable population increase in CD8 $^{+}$ T $_{SCM}$ cells as compared to baseline samples, and marginally restored CD8 $^{+}$ T cell frequencies.

Conclusion

Metabolic properties guide T cell memory differentiation and fitness. There exists potential to manipulate this mechanism and select the most metabolically fit T cell population for adoptive cell therapies. In addition to answering fundamental questions of CAR T cell biology, the combination of Venetoclax and CAR T cell therapy may provide a solution to the observed clinical gap, in which CAR T therapy as a single agent does not afford favorable response rates in the context of aggressive and R/R B-NHLs.

Disclosures Hernandez-Ilizaliturri: *ADC Therapeutics:* Consultancy; *Dava Oncology:* Consultancy; *Novartis:* Consultancy; *Amgen:* Consultancy; *Collectar:* Consultancy; *Gilead:* Consultancy; *Kite:* Consultancy; *Incyte/Morphosys:* Consultancy; *BMS:* Consultancy; *AbbVie:* Consultancy; *Epizyme:* Consultancy; *BioGene:* Consultancy.

OffLabel Disclosure: Bcl-2 inhibitor Venetoclax (ABT-199) is a small molecule inhibitor which is FDA-approved for the treatment of chronic lymphocytic leukemia and small lymphocytic leukemia. In this study, Venetoclax is repurposed in the context of B-cell non-Hodgkin lymphoma to improve anti-CD19 CAR-T cell fitness and quality.

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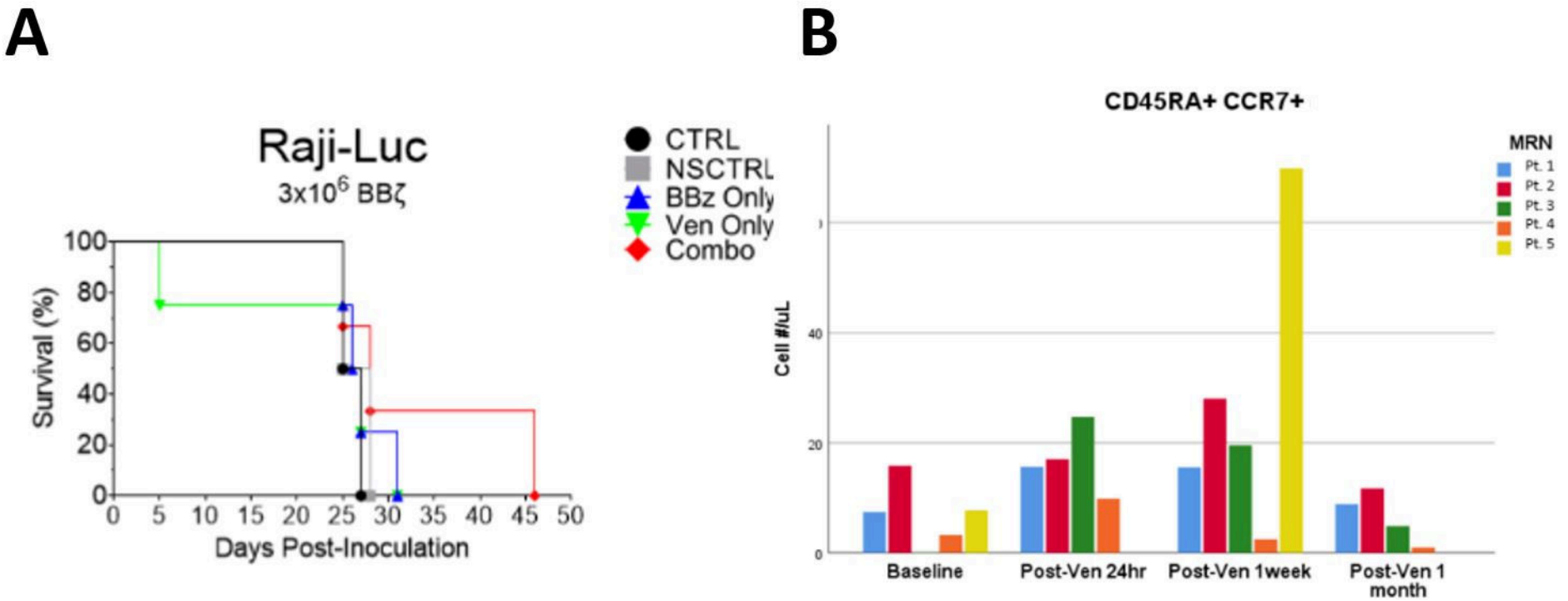

Figure One. **A)** Survival proportions of Raji-Luc inoculated 7-week-old female mice. (CTRL, vehicle control; NSCTRL, non-specific untransduced T-cell control; BBz only, 4-1BB CAR-T cells; Ven only, Venetoclax; Combo, 4-1BB CAR-T cells and Venetoclax). **B)** Frequency of circulating $CD3^{+}CD8^{+}$ T_{SCM} cells in CLL patient samples at baseline or post-treatment timepoints (24 hours, 7 days, 30 days). Reported as number of positive cells per µL of peripheral blood.

Figure 1