05. Immunometabolism

**P05.01 SENSITIZING IMMUNOTHERAPY REFRACTORY PROSTATE CANCER WITH OPTIMIZED KETOGENIC DIET REGIMEN AND EPIGENETIC REPROGRAMMING**

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**Background** Resistant to immune checkpoint blockade (ICB) therapy, especially in a few solid tumor types including advanced prostate cancer, represents a formidable clinical challenge. The ketogenic diet can enhance ICB therapy in some cancer models (prostate cancer not included yet). However, the adverse effect associated with continuous KD was also observed, demanding better mechanistic understanding and optimized regimens of using KD as an immunotherapy sensitizer.

**Materials and Methods** We developed a series of ICB-resistant murine prostate cancer cell lines from an ICB-sensitive prostate cancer cell line previously developed from the PB-Cre\(^{+}\) Pten\(^{–/–}\) p53\(^{–/–}\) Smad4\(^{–/–}\) mouse model of metastatic prostate cancer. C57BL/6 mice inoculated with dual ICB-resistant subline PPS-ICBR were treated with epigenetic modulators or specific ketone body (BHB, endogenous HDAC inhibitory metabolite) via 1,3-butanediol-admixed food. CKD and BHB supplementation delayed prostate cancer tumors as monotherapy, and both BHB and adaptive immunity are required for the anti-tumor activity of CKD. Single-cell transcriptomic and proteomic profiling revealed that the HDAC inhibitor and ketogenesis-enhanced ICB therapy was effectuated through both cancer-cell-intrinsic (upregulated MHC class I molecules) and extrinsic mechanisms (CD8\(^{+}\) T cell chemotraction, M1/M2 macrophage rebalancing, monocyte differentiation toward antigen presenting cells, and diminished neutrophils).

**Conclusions** Thus, primary CAFF-containing triple culture successfully model TAM-like phenotypes ex vivo and allow the assessment of their functional and phenotypic changes in response to treatments following a precision medicine approach.


**P05.02 INVESTIGATING THE KINETICS OF CD3 BINDING AND TRAFFICKING IN REAL-TIME**

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**Background** T-Cell Receptor complex (TCR) is an immune structure aimed at antigen recognition and initiation of immune response. It is bound to three protein dimers, known as Cluster of Differentiation 3 (CD3), whose role is related to signal transduction. Since the immune response is precisely tuned, mobilization of the TCR complex is highly regulated, as the complex undergoes several rounds of positioning on the cell membrane, endocytosis, and recycling. This study was thus aimed at understanding the kinetics of recycling and internalization of the CD3\(\varepsilon\) subunit, by targeting it with the antibody SPV-T3a. The final goal would be the development of a mathematical model of trafficking kinetics, which would not only help understanding the metabolic effect of endocytosis in inflammation processes, but also be a game changer in drug development.

**Materials and Methods** Lymphoblastic cell line Jurkat overexpressing CD3\(\varepsilon\), and K562 (negative control) were used for the experiments. Both were grown in RPMI1640 media with 10% HiFBS and 1% PeSt. The antibody SPV-T3a was labeled with either ATTO488 or the pH dependent dye pHrodo Green. Receptor trafficking was studied in real-time assays using LigandTracer Green and evaluated with TraceDrawer software.

**Results** Binding of ATTO488-SPV-T3a to CD3 receptors showed a peculiar profile, which reflected an ongoing endocytosis pattern. The plateau phase of the curve at equilibrium was replaced by a section of linear growth in which the slope was constant and independent of antibody concentration. This slope is believed to reflect the kinetics of CD3 recycling on the cell surface. Dissociation was also ligand-independent, with a biphasic profile where a first initial detachment of the complex was followed by a stable signal, given by the fluorophores accumulated in the cells. Labeling SPV-T3a with