Progress Report for Hope4ATRT – 7/10/25 - Dr. Acevedo-Duncan, University of South Florida

The central hypothesis is that a PKC-i inhibitor (ICA-1S) alone or in combination with TMZ can restrain pediatric ATRT cell proliferation and cell survival by intercepting the PKC-i/Cdk7/Cdk2 and PKC-i/Bad pathways cascade in-vitro (in petri dish).

To validate the hypothesis, we examined the effects of ICA-1S alone or in combination with TMZ on the proliferation of ATRT cells. The results of a proliferation assay after a four-day treatment period with ICA-1S, TMZ and combination helped us achieve the inhibitory concentration of the drugs that reduced the cancer cell proliferation to 50%, which was at 20μM with ICA-1S alone, 8μM with TMZ alone and 0.5μM TMZ + 20μM ICA-1S with the combination treatment.

To further validate our findings, we investigated the expression levels of proteins involved in the PKC-i/CDK-7/CDK-2 and PKC-i/BAD signaling pathways. The CDK-7 pathway is involved in cell proliferation and the BAD pathway is involved in apoptosis (cell death). The purpose of analyzing these two pathways involves the understanding of how the drugs affect the ATRT cell proliferation and if the drug treatment can lead to cancer cell death. To observe the effect of the drugs on the ATRT cells, the cells were treated for four consecutive days at 24-hour intervals with the following concentrations: 10μM ICA-1S, 20μM ICA-1S, 0.5μM TMZ, 2μM TMZ, 0.5μM TMZ + 20μM ICA-1S, and 2μM TMZ + 20μM ICA-1S. Protein expression was analyzed via SDS-PAGE and immunoblotting for protein detection.

The results demonstrated that both ICA-1S and TMZ alone reduced the levels of atypical PKC isoforms; however, the combination therapy led to a significantly greater reduction, indicating a potential synergistic effect, with the highest effect at 2μM TMZ + 20μM ICA-1S. We also observed changes in the level of CDK7, a protein that helps control the cell cycle and allows tumor cells to keep dividing, was reduced by 20μM ICA-1S and 0.5μM TMZ treatment but increased with the combination treatment. We also observed the levels of Cyclin H, which works alongside CDK7 to regulate cell division, showed moderate decrease with both individual and combination treatments. In contrast, BAD, a protein that promotes cell death, increased across all treatment conditions, which indicated that the therapies may be activating pathways that push cancer cells towards apoptosis (programmed cell death).

Nevertheless, these results represent preliminary data, and further experimentation and comprehensive analysis are required to fully validate and interpret the findings.

ICA-1S Inhibits ATRT proliferation and cell proliferation and survival signaling pathways.

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To validate the hypothesis, we examined the effects of ICA-1S alone or in combination with TMZ on the proliferation of ATRT cells. The results of a proliferation assay after a four-day treatment period with ICA-1S, TMZ and combination helped us achieve the inhibitory concentration of the drugs that reduced the cancer cell proliferation to 50%, which was at 20μM with ICA-1S alone, 8μM with TMZ alone and 0.5μM TMZ + 20μM ICA-1S with the combination treatment as indicated by Figure 1.

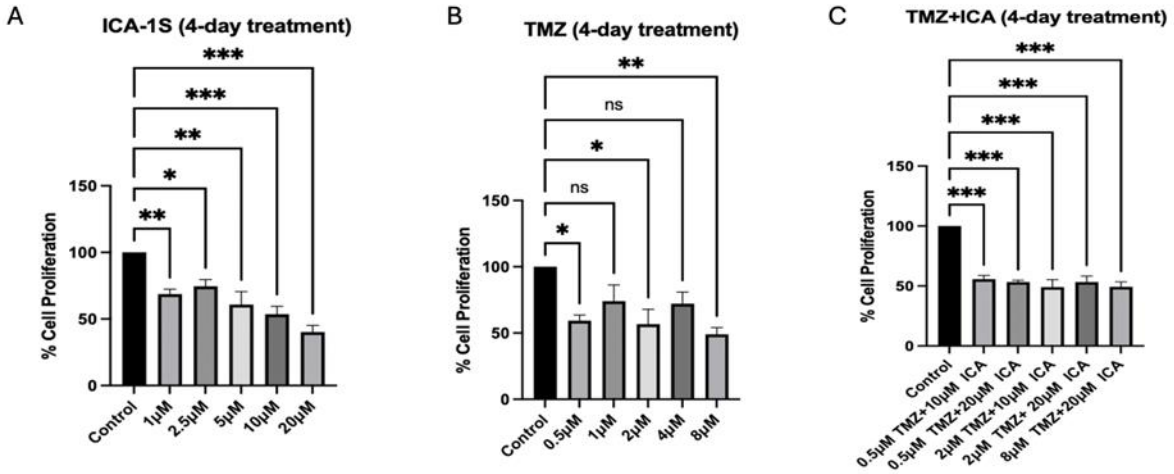

Figure 1: The dose-response curve of (A) ICA-1S (B) TMZ and (C) TMZ+ICA-1S after a 4-day treatment period. The results indicated that ICA-1S alone exhibited an IC_{50} of 20μM, TMZ alone showed an IC_{50} of 8μM, and the IC_{50} for the combination treatment was at 0.5μM TMZ + 20μM ICA-1S.

To further validate our findings, we investigated the expression levels of proteins involved in the PKC-i/CDK-7/CDK-2 and PKC-i/BAD signaling pathways. The CDK-7 pathway is involved in cell proliferation and the BAD pathway is involved in apoptosis. The purpose of analyzing these two pathways involves the understanding of how the drugs affect the ATRT cell proliferation and if the drug treatment can lead to cancer cell death. To observe the effect of the drugs on the ATRT cells, the cells were treated for four consecutive days at 24-hour intervals with the following concentrations: 10μM ICA-1S, 20μM ICA-1S, 0.5μM TMZ, 2μM TMZ, 0.5μM TMZ + 20μM ICA-1S, and 2μM TMZ + 20μM ICA-1S. Protein expression was analyzed via SDS-PAGE and immunoblotting.

The results as shown in Figure 2 demonstrated that both ICA-1S and TMZ alone reduced the levels of atypical PKC isoforms; however, the combination therapy led to a significantly greater reduction, indicating a potential synergistic effect, with the highest effect at 2μM TMZ + 20μM ICA-1S.

We also observed changes in the level of CDK7, a protein that helps control the cell cycle and allows tumor cells to keep dividing, was reduced by 20μM ICA-1S and 0.5μM TMZ treatment but increased with the combination treatment. We also observed the levels of Cyclin H, which works alongside CDK7 to regulate cell division, showed moderate decrease with both individual and combination treatments. In contrast, BAD, a protein that promotes cell death, increased across all treatment conditions, which indicated that the therapies may be activating pathways that push cancer cells towards apoptosis (programmed cell death).

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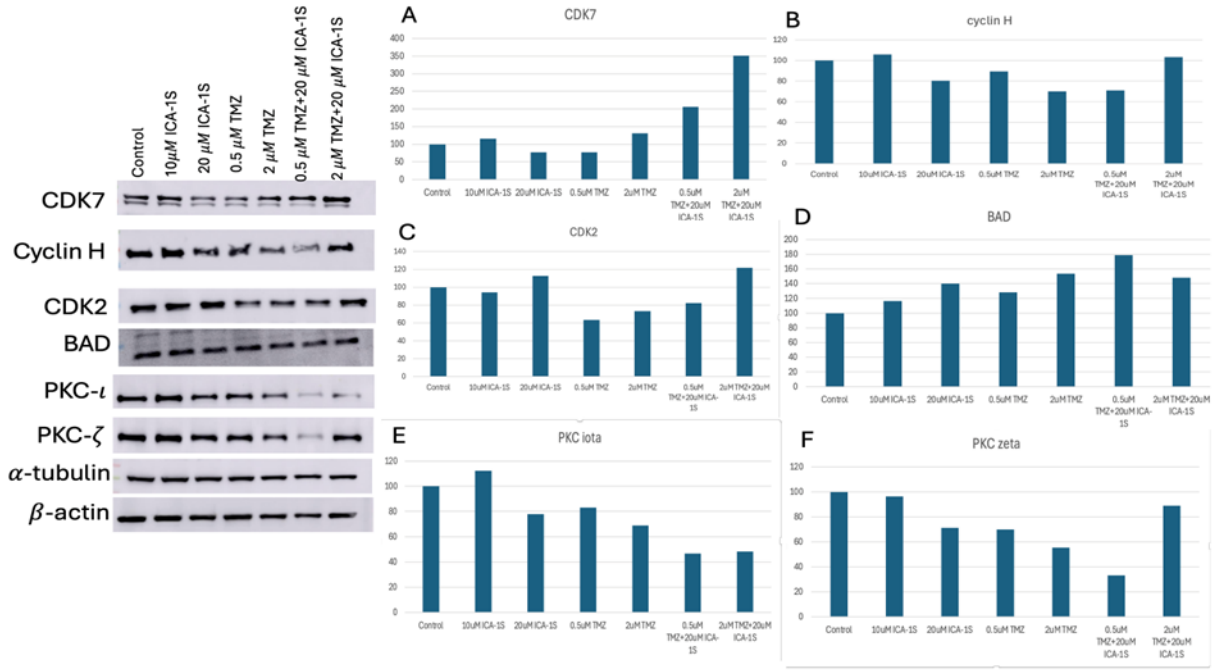

Figure 2: Western Blot analysis and the graphical representation of (A) CDK7 (B)Cyclin H (C) CDK2 (D) BAD (E) PKC-i (F) PKC-ζ