Background Although present in high numbers, T and NK cells appear functionally impaired in the renal cell carcinoma (RCC) tumor milieu, as they cannot be stimulated to degranulation and IFN-γ production. This is in part due to altered regulation of signaling downstream of the T cell receptor (TCR). Increased diacylglycerol kinase alpha (DGK-α) has been observed in T and NK cells from the RCC tumor microenvironment (TME). Ex vivo inhibition of DGK-α by the commercially available inhibitor R59022 was able to restore responsiveness to stimulation. Inhibition of DGK-α is reported to also block tumor cell growth and survival. Many T cells from RCC additionally express the immune checkpoint Programmed cell Death-1 (PD-1). Interaction of PD-1 with PD-L1 on tumor cells blocks AKT signaling and inhibits T cell function. In the clinic, blocking the PD-1/PD-L1 interaction allows tumor control in some patients; however, the majority of patients do not respond long-term. Since DGK-α acts downstream of PD-1 it may, if overactive, curb T cell function despite PD-1/PD-L1 blockade. Thus, we hypothesize that dual inhibition of PD-1 and DGK α might be required to fully unleash the T cell’s potential in the TME. Current DGK-α inhibitors are not suitable for clinical application. Therefore, we investigate alternative means using RNA interference (RNAi) to target DGK-α alone as well as in combination with PD-1.

Methods Knockdown was achieved by RNAi using INTASYL™ compounds, developed by Phio Pharmaceuticals. These compounds incorporate drug-like properties into siRNA, resulting in enhanced uptake with no need for transfection reagents. Efficacy was analyzed on mRNA and protein level by rt-qPCR, flow cytometry and Western Blot. Functional assays include cytotoxicity and cytokine production in tumor-mimicking environments.

Results Using INTASYL™ compounds, silencing of DGK-α was observed in human U2OS osteosarcoma as well as K562 erythroleukemic cells. PD-1 knockdown was achieved in human T cells isolated from peripheral blood mononuclear cells (PBMC). Synergy of DGK-α and PD-1 knockdown was tested in tumor-minicking in vitro systems using T cell/tumor cell co-cultures at high tumor cell density where T and NK cells become functional suppressed as observed in the TME.

Conclusions Strong activity of specific T and NK cells is necessary for tumor control. Dual targeting of PD-1 and DGK-α may be required to fully enable T and NK cell reactivity in the TME. Self-delivering RNAi technology represents a promising approach to targeting intracellular immune checkpoints such as DGK-α, in addition to PD-1 inhibition.

REFERENCES

