compared to untreated controls in the presence of dsDNA or 2'3'-cGAMP (~2,000 pg/ml, P < 0.001).

Conclusions We provide evidence that methylation silencing of cGAS and STING is not only a notable mechanism of STING signaling dysfunction in melanoma, but also plays a role in tumor antigen presentation and recognition by TIL. Collectively, these observations argue that targeting epigenetic loss of STING signaling in melanomas should be considered as a strategy to improve the efficacy of clinical interventions using T cell-based immunotherapies.

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599 NEW CHECKPOINTS CONTROLLING FUNCTION OF CYTOTOXIC LYMPHOCYTES INFILTRATING HUMAN CARCINOMA

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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.[1] [2] Inhibition of DGK-α is reported to also block tumor cell growth and survival.[3] [4] 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 is tested in tumor-mimicking 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.

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600 IN VITRO ANTICANCER AND IMMUNOMODULATORY ACTIVITIES OF NBT-167, A DIMER OF RESVERATROL

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Background Resveratrol (3,4',5-trihydroxystilbene), a stilbenoid isolated from many species of plants, is widely known for its antioxidative, anti-inflammatory, immunomodulatory and anticancer activities. Recently, novel resveratrol oligomers have been isolated from various plants; their diverse structures are characterized by the polymerization of two or more resveratrol units. Little is known regarding the anticancer and immunomodulating activities of these oligomers. In this study, we designed in vitro models to compare resveratrol side by side with its natural dimer NBT-167 for their anticancer and immunological activities.

Methods We isolated resveratrol and its dimer (NBT-167) from plants. The potency of the compounds was compared side by side using cancer cell survival assays and immunological assays with various types of human cells including cancer cell lines, PBMCs and enriched NK, gamma delta T cells, THP-1 monocytic cells, HL-60 promyelocytic leukemia cells as well as mouse RAW264.7 macrophages.

Results NBT-167 was found to be more potent than resveratrol in inhibiting growth of various cancer cells and modulation of cytokine production from anti-IgM, LPS, PHA or SEB stimulated PBMC. Both compounds similarly enhanced IL-2 stimulated NK and gamma delta T cell killing activity against K562 cells and modulated nitric oxide production from LPS/IFN-g induced RAW264.7 macrophages and phagocytotic activity of HL-60 cells. NBT-167 was slightly more potently

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