Background: Mesothelin (MSLN) is recognized as a relevant tumor-associated antigen for cancer immunotherapy, because of its overexpression on various solid tumors, including mesothelioma, pancreatic, lung, gastric and ovarian carcinoma. However, an anti-MSLN monoclonal antibody (mAb), amatuximab, has demonstrated only limited efficacy in clinical trials. It has been already demonstrated that the targeting of a membrane-distal domain of an antigen with a mAb is suboptimal at inducing Fc-related effector functions. As amatuximab targets a membrane-distal domain of MSLN, we investigated whether mAbs targeting different epitopes would bestow a better efficacy. Furthermore, in order to incorporate novel modalities to enhance tumor-killing, we have paired these MSLN targeting arms with an anti-CD47 arm to generate bispecific antibodies (bsAb). Indeed, the ‘don’t eat me signal’ CD47 is a promising target in cancer and therapeutic blockade has recently showed clinical evidence of efficacy. Therefore, we investigated the contribution of a CD47 arm and the impact of the different anti-MSLN targeting arms on the tumoricidal activities of CD47xMSLN bsAbs.

Materials and Methods: A panel of anti-MSLN mAbs and CD47xMSLN bsAbs carrying the same anti-CD47 arm and different anti-MSLN arms were generated and characterized for their epitope specificity. Their tumor cell killing efficacy in vitro and in vivo was analyzed using cell-based assays, xenograft models and various MSLN+ human malignant cell lines originated from different tissues (e.g., lung, gastric and hepatic origin).

Results: Our analyses revealed more than 20 distinct cell types in human breast cancer and normal tissues. Cell populations, biomarker expression and cellular spatial distributions differed distinctly between cancerous and normal breast tissues. Differences were robust, repeatedly observed and indicative of altered cellular milieu in normal versus cancerous breast tissues.

Conclusions: Collectively, these data establish CODEX® as a readily deployable and practical tool for spatially-resolved, highly multiplexed biomarker analysis of human FFPE samples.

Disclosure Information: O. Brauch: A. Employment (full or part-time); Significant; Akoya Biosciences. S. Basak: A. Employment (full or part-time); Significant; Akoya Biosciences. M. Gallina: A. Employment (full or part-time); Significant; Akoya Biosciences. W. Lee: A. Employment (full or part-time); Significant; Akoya Biosciences. J. Kim: A. Employment (full or part-time); Significant; Akoya Biosciences. C. Hempel: A. Employment (full or part-time); Significant; Akoya Biosciences. E. Williams: A. Employment (full or part-time); Significant; Akoya Biosciences. O. Shang: A. Employment (full or part-time); Significant; Akoya Biosciences. B. Cheung: A. Employment (full or part-time); Significant; Akoya Biosciences. J. Kennedy-Darling: A. Employment (full or part-time); Significant; Akoya Biosciences.
discussed, was restored ex vivo. A correlation of high DGK-α and loss of function was also observed in an experimental mouse model of adoptive therapy where CAR T cells that had lost their activity after infiltrating into solid tumors were found to have increased DGK-α. Blockade of the Programmed cell death protein 1 (PD-1) with monoclonal antibodies is used in the clinic enabling some patients to achieve tumor control. However, not all patients respond. DGK-α activity is positioned downstream of PD-1 and should, if overactive, curb T cell function even if PD-1 inhibition is released. 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 investigated alternative means using an RNA interference (RNAi) approach to target DGK-α alone as well as in combination with PD-1 in T and NK cells.

**Material and Methods**

Knockdown is performed by RNAi using INTASYL™ compounds developed by Phio Pharmaceuticals. INTASYL™ compounds incorporate drug-like properties into the siRNA, resulting in enhanced uptake in the presence of serum with no need for further transfection reagents. Knockdown is analyzed by RT-qPCR and flow cytometry. Functional assays include cytotoxicity, degranulation and cytokine production in tumor mimicking environments.

**Results**

A tumor mimicking in vitro system was developed which allows for the demonstration of functional restoration or prevention of functional loss of cell activity. Using T cell/tumor cell co-cultures at high tumor cell density, functional suppression could be induced in T and NK cells comparable to those observed in the TME. Testing of DGK-α targeting INTASYL™ compounds, silencing of DGK-α was observed in human U2OS osteosarcoma cells. Using a fluorescently labeled compound, highly efficient transfection of human primary immune cells was seen. Combinations of PD-1 and DGK-α targeting compounds are being tested and evaluated for synergism in experimental models.

**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. Current DGK-α inhibitors do not exhibit the desirable pharmacokinetic/pharmacodynamic (PK/PD) properties for clinical development. The tested self-delivering RNAi technology represents a promising approach to targeting intracellular immune checkpoints such as DGK-α.

**REFERENCE**


**Disclosure Information**

A.S. Herbstritt: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Phio Pharmaceuticals. C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; Phio Pharmaceuticals. P.U. Prinz: None. M. Maxwell: A. Employment (full or part-time); Significant; Phio Pharmaceuticals. M. Kadiyala: A. Employment (full or part-time); Significant; Phio Pharmaceuticals. D. Yan: A. Employment (full or part-time); Significant; Phio Pharmaceuticals. E. Noessner: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Phio Pharmaceuticals. C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; Phio Pharmaceuticals.