Poster 292

Precision Cut Cancer Tissue Slices derived from cancer patients as a tool for the investigation of immune-modulatory compounds

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Introduction

The goal of personalized medicine is to provide individual patients with the most appropriate treatment. This approach strongly depends on extensive characterization of individual tumors and their sensitivity to therapeutics. We previously have shown that our drug testing platform based on Precision Cut Cancer Tissue Slices (PCCTS) is applicable to analyze individual responses of patients to defined compounds. In the context of immunotherapy, the testing of immune-modulating compounds such as immune checkpoint modulators or bispecific antibodies gained in importance. In this study, we investigated the effects of OKT3 as well as Nivolumab on cancer tissue slices especially in respect of protein expression changes and cytokine release.

Methods

Samples: Vital tumor tissue from colorectal cancer (CRC) and Non Small Cell Lung Cancer (NSCLC) patients were collected immediately after resection according to Indivumed's Standard Operating Procedure. Informed consent was obtained from all patients.

Preparation of Precision Cut Cancer Tissue Slices (PCCTS): Vital tumor tissue from CRC patients was used as starting material for the preparation of PCCTS. Therefore, fresh tumor tissues were cut into 500 µm slices using a Krumdieck™ tissue slicer (TSE Systems).

Cultivation and drug treatment: PCCTS were cultivated in a supplemented RPMI 1640 tissue culture medium in 24 well plates. For drug treatment PCCTS were pre-cultured for one hour. Subsequently, PCCTS were incubated for 18h with and without OKT3®, (Muromonab), a therapeutic antibody against CD3 or the checkpoint inhibitor Nivolumab. For each condition three PCCTS were treated. After defined time points, slices were frozen, and the supernatants were collected. In addition, at timepoint T0 after pre-cultivation a set of slices was formalin fixed and paraffin embedded for analysis of T-cells.

Simple Western Size (SWS): Protein expression was examined by Simple Western Size technology. Simple Western Size assays were performed according to our Standard Operating Procedure and were run on the Peggy Sue instrument.

Meso Scale Discovery (MSD): The analysis of cytokines in the supernatants of tissue cultures was performed using the validated ten-plex proinflammatory panel from MSD. Supernatants for each condition were pooled and analyzed.

Immunohistochemical (IHC) Staining: Anti-CD3 was implemented on the DISCOVERY XT/Benchmark Ultra staining platform (Ventana). Image analysis was conducted using Axio Scan.Z1, Zeiss. The three PCCTS for each condition were pooled and embedded in one FFPE block.

Figure 1: Schematic illustration of the technical workflow of the "Precision Cut Cancer Tissue Slice" platform.

Experimental Setup

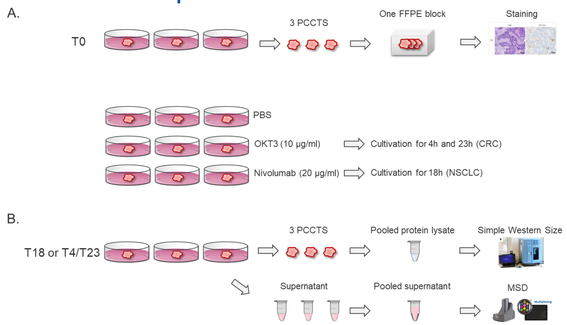

Figure 2: Schematic illustration of experimental setup for **A.** Timepoint T0 after pre-cultivation. PCCTS were formalin fixed and paraffin embedded for subsequent IHC analysis. **B.** Experimental setup at 4h (T4) and 23h (T23) for CRC cases and at 18h (T18) for NSCLC case. PCCTS and supernatants were collected for Simple Western Size analysis and MSD analysis.

Immune cell infiltration in CRC tissue slices

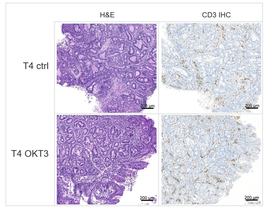

Figure 3: H&E and anti-CD3 IHC of human FFPE colorectal cancer (CRC) tissue. A strong membranous anti-CD3 staining of immune cells was detected.

Table 1: Evaluation of anti-CD3 IHC of CRC tissue samples.

Tissue type	Tumor content [%]	Infiltration of CD3-positive cells					
		Tumor region					
		HPF1	HPF2	HPF3	HPF4	HPF5	Average
CRC	30	165	183	98	208	252	186
CRC	25	143	127	232	272	243	203
CRC	40	64	110	156	241	76	129

The number of CD3-positive immune cells per High-Power Field (HPF) were quantified in the tumor region and in the peritumoral stroma. Five HPFs were evaluated per sample and region, and the average numbers of CD3-positive immune cells per HPF were calculated. CD3-positive cells were detected in all CRC tissue samples, showing averages from 129 to 203 CD3-positive cells per HPF.

Cytokine release upon OKT3 treatment

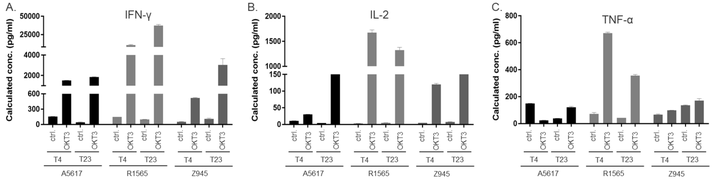

Figure 4: Cytokine secretion of untreated and OKT3 treated PCCTS from three CRC patients. PCCTS were treated with 10 µg/ml OKT3 for 4 and 23 hours. Cytokine secretion was analyzed in supernatants of tissue cultures using the validated ten-plex proinflammatory panel from MSD. Shown is the mean value with standard deviation of cytokines in pg/ml compared to the untreated control.

Signaling pathway analysis upon Nivolumab treatment

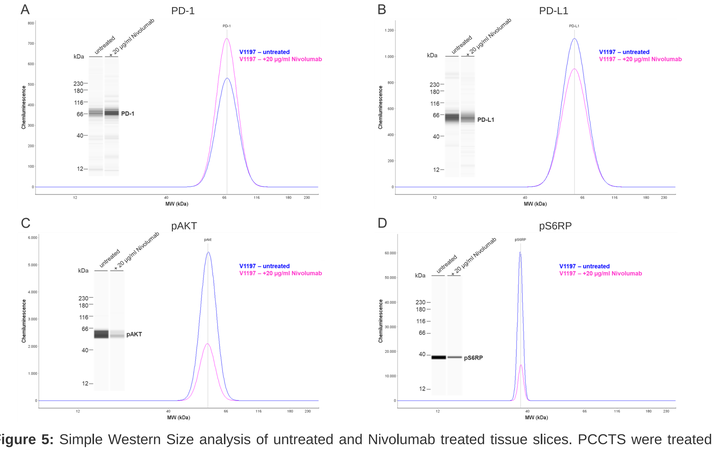

Figure 5: Simple Western Size analysis of untreated and Nivolumab treated tissue slices. PCCTS were treated with 20 µg/ml Nivolumab for 18 h. Protein expression of precision cut cancer tissue slices was analyzed using anti-PD-1 (A), anti-PD-L1 (B), anti-pAKT (C) and anti-pS6RP (D) antibodies. Shown is both, the lane view and electropherogram view of software generated peak fit.

Cytokine release upon Nivolumab treatment

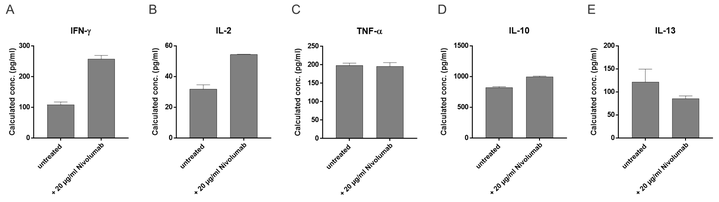

Figure 6: Cytokine secretion of untreated and Nivolumab treated PCCTS from one NSCLC patient (V1197). PCCTS were treated with 20 µg/ml Nivolumab for 18 hours. Cytokine secretion was analyzed in supernatants of tissue slices using the validated ten-plex proinflammatory panel from MSD. Shown is the mean value with standard deviation of cytokines in pg/ml compared to the untreated control.

Conclusion and Summary

- OKT3 treatment induced cytokine secretion into the supernatant. Especially high levels of IFN-γ, IL-2, and TNF-α were detectable.
- Nivolumab treatment induced secretion of IFN-γ and IL-2.
- Upon treatment with Nivolumab, expression of pAKT and pS6RP was decreased.
- The model of PCCTS is suitable for pre-clinical evaluation of immunomodulatory compounds.