Poster 2128

Simple Western™ Size (SWS) in combination with precision cut cancer tissue slices: An excellent patient-derived platform to support drug development

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Introduction

The goal of personalized medicine is to provide individual patients with the most appropriate treatment. 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 Nivolumab on cancer tissue slices especially in respect of protein expression changes and cytokine release.

Methods

Samples: Vital tumor tissues from 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 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. PCCTS were incubated for 18 h with and without the checkpoint inhibitor Nivolumab. For each condition three PCCTS were treated. After defined time points, slices were frozen in liquid nitrogen or slices were formalin fixed and paraffin embedded. The supernatants were collected. **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-PD-L1 and anti-PD-1 were implemented on the DISCOVERY XT/ Benchmark Ultra staining platform (Ventana). **Multiplex Immunohistochemical (mIHC) Staining:** For the detection of CD3, CD8, FOXP3 and pan-Cytokeratin (pCK) a tyramide signal amplification (TSA)-based fluorescent mIHC assay was implemented on the Leica BOND RX automated staining platform. Image analysis was conducted using Axio Scan.Z1, Zeiss. **Simple Western Size (SWS):** Protein expression was examined by Simple Western Size (SWS) technology. SWS assays were performed according to our Standard Operating Procedure and were run on a Peggy Sue instrument.

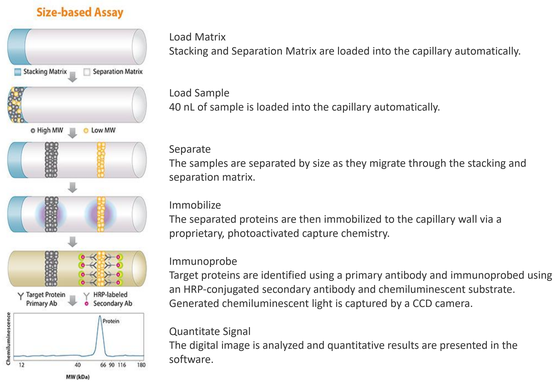

Figure 1 Schematic illustration of the technical workflow of the SWS technology (https://www.proteinsimple.com/).

Precision Cut Cancer Tissue Slices (PCCTS) Platform

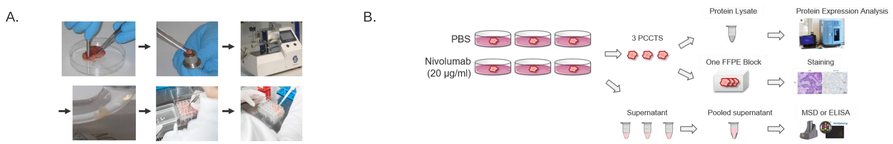

Figure 2 Schematic illustration of the technical workflow of the "Precision Cut Cancer Tissue Slice" platform (A) and of experimental setup (B). After 18 h of cultivation PCCTS were collected for either Simple Western Size or IHC analysis. Supernatants were collected for MSD analysis.

Signaling Pathway Analysis upon Nivolumab Treatment

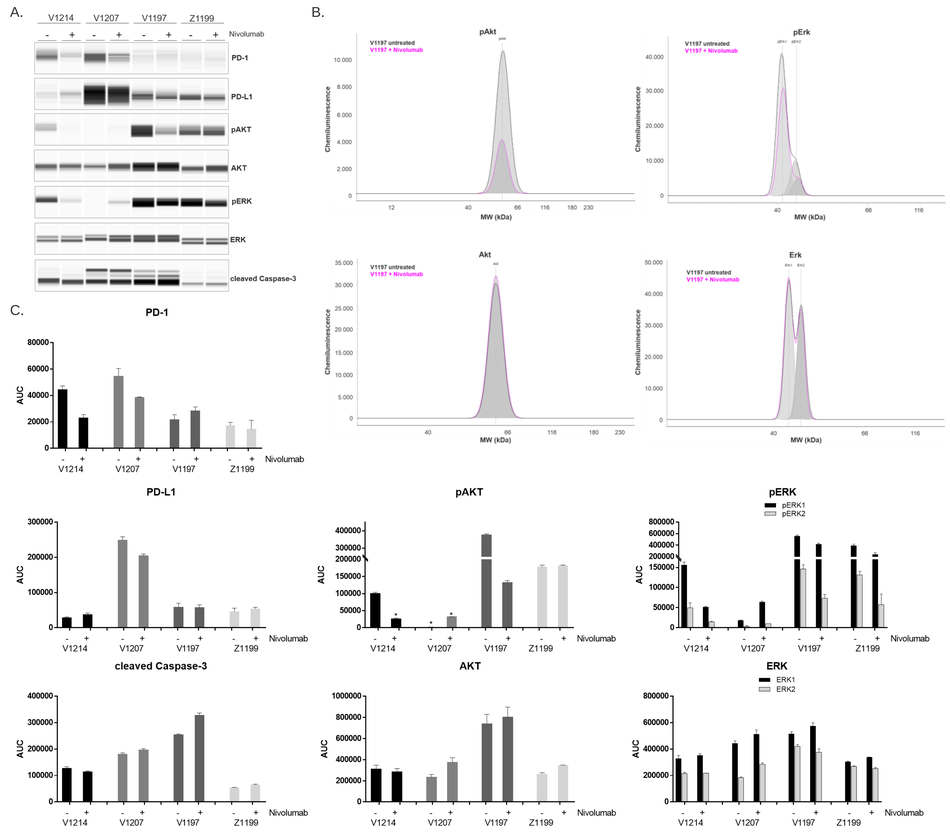

Figure 3 Simple Western Size analysis of untreated and Nivolumab treated tissue slices. PCCTS were treated with 20 µg/ml Nivolumab for 18 h. Protein expression of signaling molecules was analyzed in protein lysates of PCCTS. Shown is the lane view (A), the electropherogram view of selected targets of the software generated peak fit for quantification (B) and quantification of all targets measured in duplicates (C).

Immune Cell Infiltration in NSCLC Tissue Slices

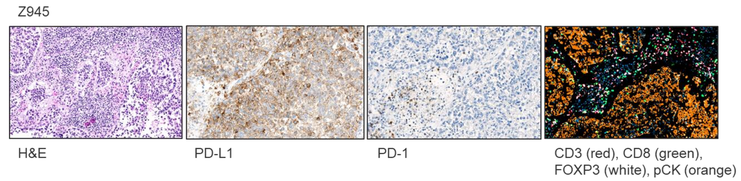

Figure 4 H&E staining, anti-PD-L1 and anti-PD-1 IHC staining as well as anti-CD3, anti-CD8, anti-FOXP3 and anti-pCK mIHC staining of formalin-fixed and paraffin embedded PCCTS of NSCLC patient Z945 after 18 h of cultivation. A strong immune cell infiltration was observed.

Cytokine Release upon Nivolumab Treatment

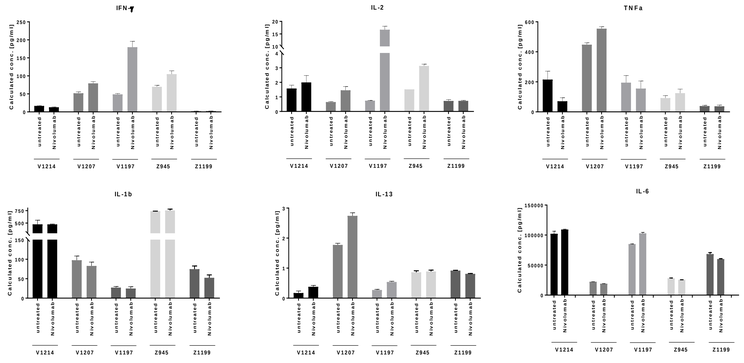

Figure 5 Cytokine secretion of untreated and Nivolumab treated PCCTS from NSCLC patients. PCCTS were treated with 20 µg/ml Nivolumab for 18 hours. Cytokine secretion was analyzed in supernatants of tissue slices using the validated ten-plex proinflammatory panel from MSD. Shown is the mean value with standard deviation of cytokines in pg/ml compared to the untreated control.

Conclusion and Summary

- Nivolumab treatment induced secretion of IFN-γ and IL-2 in distinct NSCLC cases
- Upon treatment with Nivolumab, expression of pAKT and pERK was decreased and expression of cleaved Caspase-3 was induced in distinct NSCLC cases
- Immune cell infiltration was detected by IHC and mIHC in PCCTS
- The model of PCCTS is suitable for pre-clinical evaluation of immunomodulatory compounds. Different read-out methods for downstream analysis including high sensitive, precise and accurate detection of proteins in minute amounts of tissue by SWS, by MSD as well as IHC and mIHC can be used. Multicolour flow cytometry analysis from viable single cell suspensions of PCCTS and genomic analysis are further options to evaluate effects of immunomodulatory compounds

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