Transcriptomics Analysis in a Renal Cancer Model Enables the Deconvolution of Additive and Synergistic Effects of Six Different Standard of Care Compounds with anti-PD-1 Treatment

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1 INTRODUCTION

Identifying how to optimally combine immunotherapies with other available anti-cancer therapies is a major challenge in oncology. Traditionally, only one potential combination can be examined per mouse in a systemic dosing study, and large cohorts are needed to draw statistically significant conclusions on its efficacy. We have utilized an implantable microdevice (IMD) performing microdosing to measure intratumor drug responses and anti-tumor immunity for 20 agents in parallel. For each of the agents, local tumor response is measured by cyclical immunofluorescence for deep cellular response phenotyping. This approach is combined with systemic administration of checkpoint inhibitors to examine whether local immunogenic cell death (ICD) induced by a given drug microdose potentiates the immunotherapy's anti-tumor effect. The measurements were performed in a humanized mouse model of renal cancer, patient derived xenograft (PDX) RXF488. The PDX is derived from a 68 year old male patient suffering from clear cell renal carcinoma. RXF488 was implanted subcutaneously in 30 NSG mice. Animals were stratified into 6 groups with n= 4-6. Humanization was performed by the intravenous injection of 5x10e6 human peripheral blood mononuclear cells (PBMC) prior to the first treatment. Systemic anti-PD1 treatment was applied in the presence and absence of the microdevice. Control groups received the microdevice in the presence or absence of PBMC. Beside the immuno-histological examination of the tumor tissue, flow cytometry (FC) was performed on bone marrow, spleen and tumor tissue to determine infiltration of human immune cells. The most active compounds in the IMD screening were confirmed in a systemic treatment experiment.
Spatial transcriptomics for a set of 1825 genes using the NanoString technology was performed on selected samples to achieve a deeper understanding of the compound effects. The generated data was used for clustering and GO enrichment analysis to identify pathways modulated by the treatment.
This presents a workflow, suitable for a complex study design, testing multiple compounds with and without systemic treatments in a humanized animal model, with a high-dimensional output dataset.

Figure 1. Microdevice technology, image analysis workflow and examined compounds

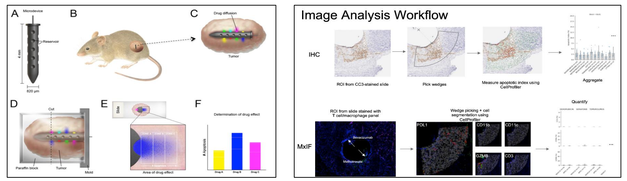


Table 1. Panel of drugs included in microdevice study. IHC regions from compounds in **bold** were used for transcriptomics.

Axitinib	Bevacizumab	**Cisplatin**	**Doxorubicin**	LTT462	**LXH254**
Methotrexate	**Oxaliplatin**	**Sorafenib**	**Sunitinib**	Temsirolimus	

2 RESULTS

FC analyses revealed no influence of the treatment on the human immune cells in bone marrow and spleen. Several agents showed a significant increase in apoptosis induction when aPD1 was added. Amongst others Sorafenib and Oxaliplatin. Cisplatin showed no change in the apoptotic index.

IHC staining in the areas around the microdevice has shown high expression of granzyme B for the vehicle treated cisplatin and oxaliplatin groups, that was correlated to high levels of the immune cell markers CD8a and CD3. Another difference in immune cell infiltration profiles could be observed for markers of myeloid cells, that have shown elevated invasion rates for the aPD1 group, compared to control.

An overview of the transcriptomics dataset was generated using several clustering approaches. The overall structure of the data reveals three clusters using the R umap package, the control group clustering separately from most of the treatment groups.
Using hierarchical clustering allows to further subdivide the clusters, but, as not all combinations were tested for this approach, no definite mapping of clusters and treatments could be performed.
Running enrichment analysis on genes that were highly up- or downregulated in comparison to the corresponding control revealed gene signatures, that corresponded to distinct mechanisms of action for three of the tested compounds.
A clear downregulation in the predefined ICD gene subset could be observed for oxaliplatin at the lower dose range.
We were able to confirm this using the reactome enrichment analysis on subsets of downregulated genes.

Figure 2. Data from IHC stainings for apoptotic areas and immune cell infiltration markers

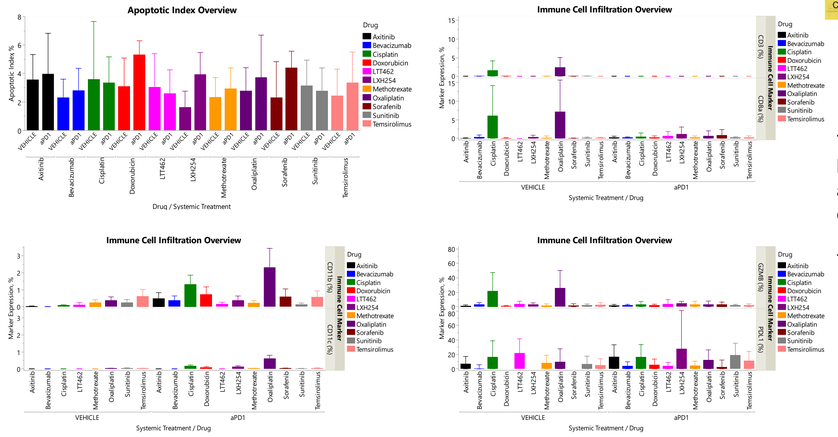


Figure 3. UMAP clustering of the 1825-gene dataset

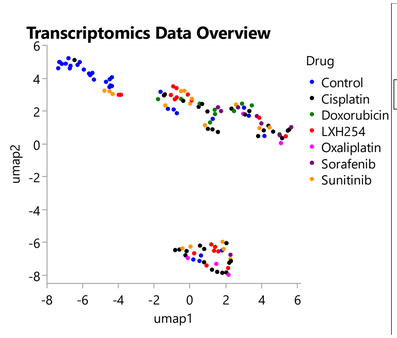


Figure 4. Heatmap of gene expression for the ICD gene subset

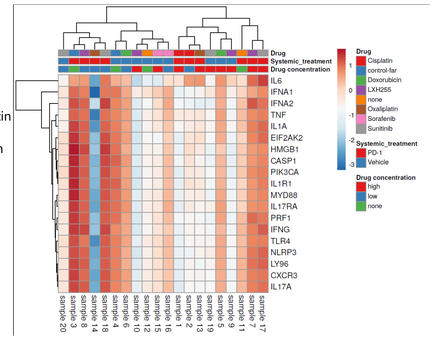
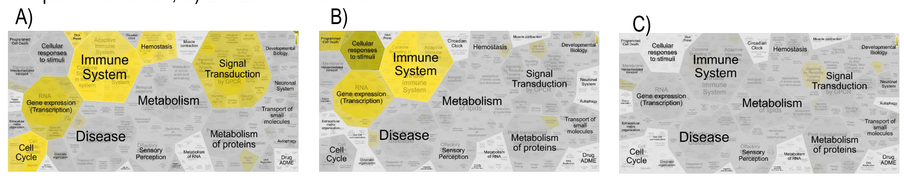


Figure 5. Gene enrichment analysis using the reactome pathway browser: A) Cisplatin with aPD1, B) Oxaliplatin with aPD1, C) LXH254 with vehicle



3 CONCLUSION

The usage of implantable microdevices for testing of multiple different compounds within a single tumor allows high-throughput screening of multiple compounds in an in vivo setting avoiding toxicity issues. The methodology allows to maximize data yield from a single experiment, by combining standardized readouts, such as flow cytometry, or immunohistochemistry with rather novel methods, such as spatial transcriptomics.

The presented addition to the previously reported workflow allows to gain further insights into the mechanisms of action of the test compounds, without the need to increase sample sizes or to enroll additional animals into the study.

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