Using Mass Spectrometry Imaging to predict patient's treatment response from bladder resections

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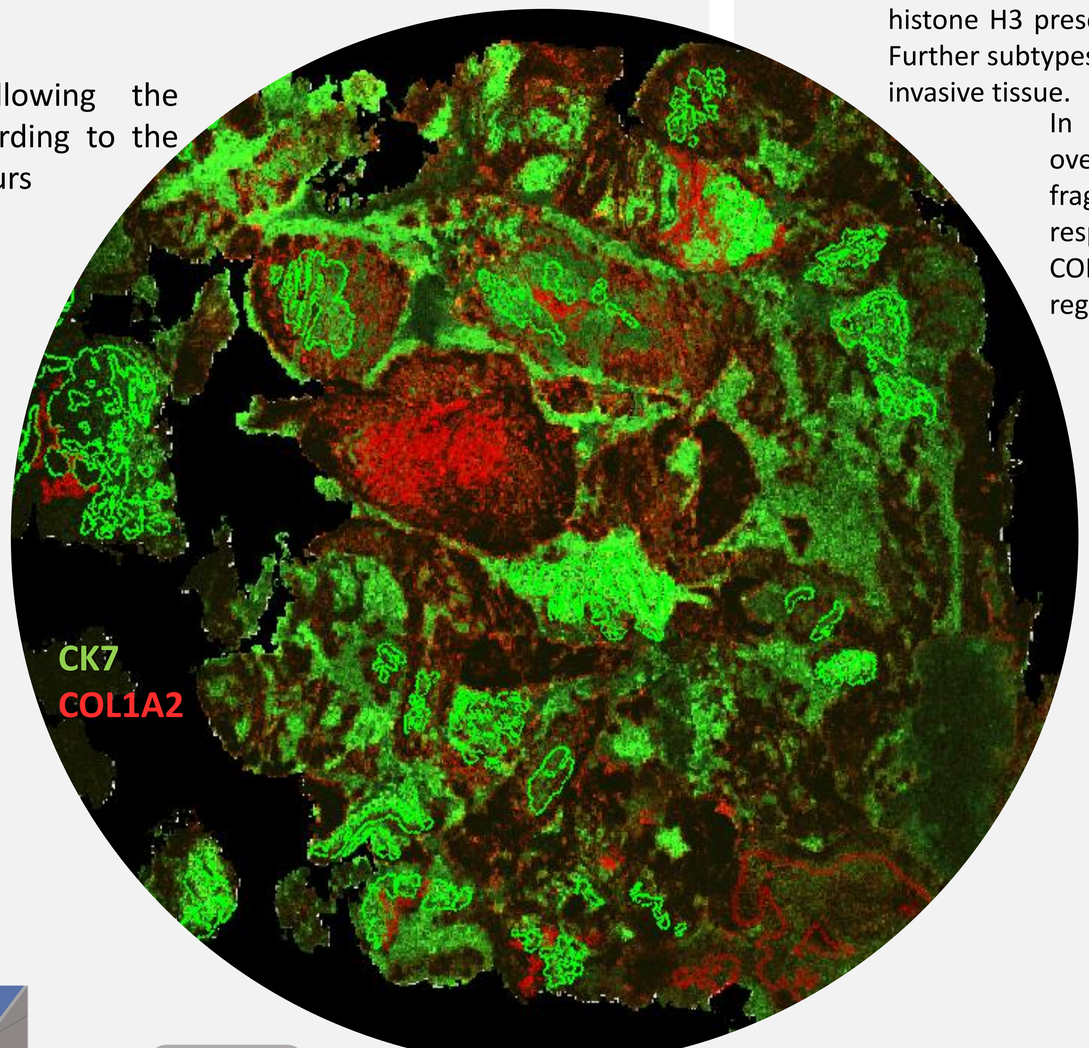
Introduction

Urothelial bladder cancer is responsive to immune checkpoint inhibitor (ICI) treatment. However, when monotherapy is applied only a minority of patients respond. Therefore, combining ICI treatment with other modalities such as radiation together with molecular preselection of patients who are likely to respond are potential avenues to optimize ICI regimens further.
This clinical study combined radiation with ICI treatment in the neoadjuvant setting as a therapeutic option in bladder cancer. We employed mass spectrometry imaging (MSI) to evaluate pre-therapeutic specimen of patients undergoing this treatment approach to identify molecular features that, upon diagnosis, can predict the patient's response to this treatment. The outcome was compared with the results from DNA/RNA sequencing.

Methodology

- Samples (n=48) were evaluated following the pathology guidelines and stratified according to the response evaluation criteria in solid tumours
- DNA/ RNA sequencing (TSO500 Illumina) on consecutive sections
- Deparaffinization and re-hydration
- Tryptic digestion at 50°C
- α-CHCA matrix application (HTX Imaging, TM-Sprayer)
- MSI data acquisition - RapifleX MALDI-TOF (Bruker), *m/z* = 600-3200
- H&E staining
- Histological annotation of viable invasive and non-invasive carcinoma regions
- Data analysis using SCiLS Lab Pro



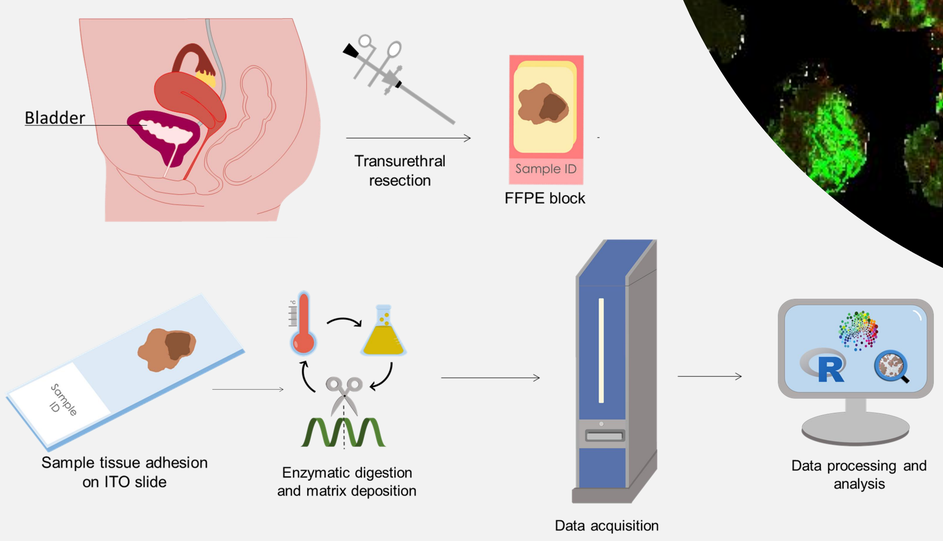


Figure 1. The bladder resection specimen were paraffin/fixed, paraffin embedded. From these blocks a 4 µm section was cut and adhered to a glass slide. After tryptic digestion at 50°C, α-CHCA was applied (TM-sprayer, HTX Technologies LLC). The peptide content was measured using a rapifleX (Bruker) and data analysis was performed using SCiLS Lab Pro.

Results

RNAseq and Infinium MethylationEPIC array combined analysis

Pathway	Genes	Description
Module_47	SGCE CXCR4 F2R VAV3	ECM and collagens
Benporath_es_with_H3K27Me3	HSPA1A VAV3 HSPA1L COL25A1 FBN2 F2R	Set 'H3K27 bound': genes posessing the trimethylated H3K27 (H3K27me3) mark in their promoters in human embryonic stem cells, as identified by ChIP on chip.

Table 1. Gene Set Enrichment Analysis (GSEA)

Results

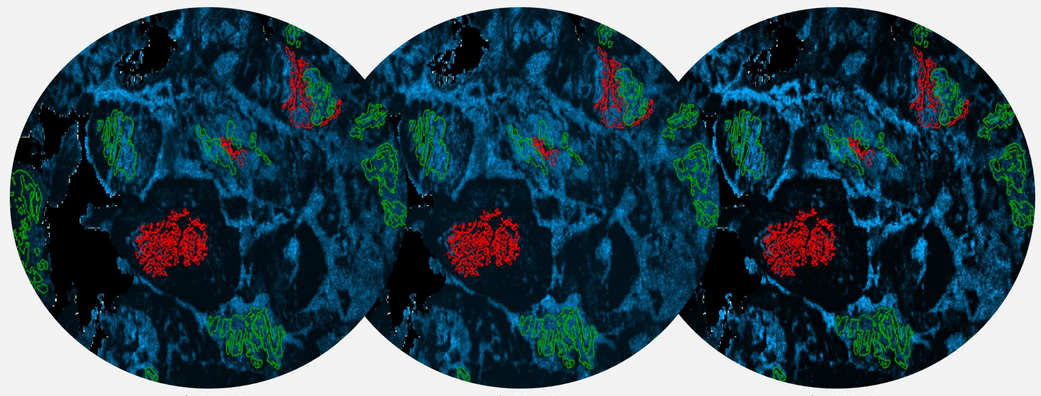


Figure 2. On tissue distribution of different fragments derived from Histone H3, showing higher correlation with non-invasive tissue (green) when compared with invasive regions of the tissue (red). This correlation is further supported by the average intensity of each region, as shown in figure 3.

From the receiver operating characteristic – area under the curve (ROC-AUC) analysis of the different tissue subgroups, overexpression of histone H3 presented a consistent correlation with non-invasive tissue. Further subtypes of histone were also found to be overexpressed in non-invasive tissue.

In addition, also *m/z* 1104.57 and *m/z* 1406.71 were overexpressed in non-invasive tissue. These peptides are fragments of KRT7 (SAYGGPVGAGIR and SIHFSSPVFTSR, respectively). On the other hand, an overexpression of COL1A1 and COL1A2 was identified in the invasive tumor regions (Figure 4).

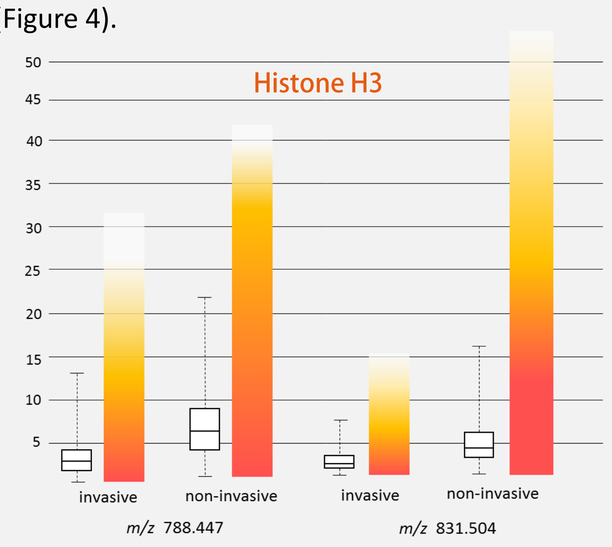


Figure 3. Intensity boxplot showing the distribution of the different fragments of histone H3 in the invasive and non-invasive tumor regions

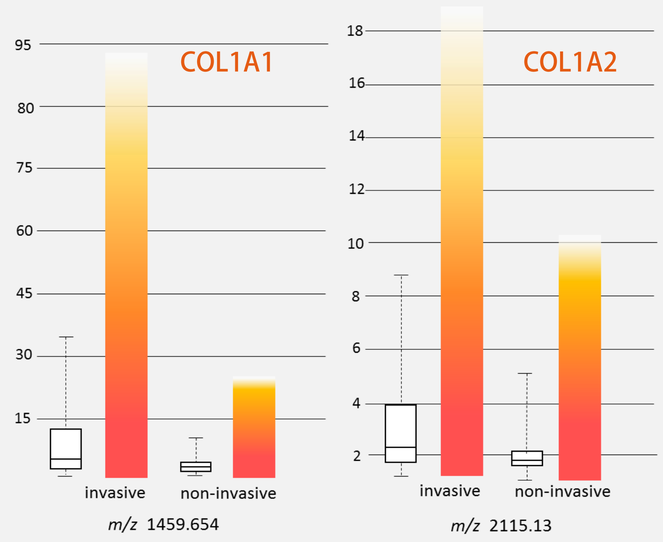


Figure 4. Intensity boxplot showing the distribution of fragments of collagen in the invasive and non-invasive tumor regions.

Patients who did not respond to the treatment exhibited elevated expression levels of COL1A1 and COL1A2, with notably increased expression in the invasive regions of the tumor, as indicated in Table 2. Additionally, in the non-invasive regions, there was a significantly higher expression of collagens observed in the non-responder group.

m/z ± 99.2 ppm	AUC-ROC	Tentative ID	Sequence (Modification)
1546.8	0.650	Collagen α-1(I) chain	R.GETGPAGPAGPVGPVGAR.G
1477.76	0.645	Collagen α-2(I) chain	R.GLHGEFGLPGPAGPR.G
2959.37	0.634	Collagen α-2(1) chain	G.PPGAAGAPGPQGFQGPAGEPGEPGQTGPAGA.R (Hydroxylated)
2705.27	0.624	Collagen α-1(1) chain	G.FSGLQGPPGPPGSPGEQGPSGASGPAGP.R (Hydroxylated)
2869.44	0.623	Collagen α-1(1) chain	G.LTGPIGPPGPAGAPGDKGESGPSGPAGPTGA.R (Hydroxylated), Oxidation (P)
1706.81	0.622	Collagen α-1(I) chain	K.DGEAGAQGPPGPAGPAGER.G

Table 2. Top features overexpressed in invasive tissue from the patients that did not have a positive response to the treatment, calculated from ROC-AUC analysis.

Conclusion and Outlook

In this study, we used MALDI-MSI to identify predictive markers in tissue samples form patients undergoing a novel treatment modality by analyzing the proteomic content of transurethal bladder resection specimen from two distinct patient groups: those who exhibited a complete pathological response and those who did not respond and presented residual tumor after treatment.
The results obtained from both MALDI-MSI and genetic analysis consistently highlighted the involvement of specific underlying pathways. Notably, these pathways included keratin-associated pathways and genes associated with histone methylation, which appeared to be favorable for the effectiveness of this treatment modality. Conversely, non-responders demonstrated a higher expression of collagen.
Mass spectrometry imaging of different analytes, namely *N*-Glycans, might enlighten further pathways that could stronger support our hypothesis.

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