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Sustainable healthcare: A future for all

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Spatial Profiling Identifies Therapeutic Targets in Rare Instance of Nested Variant of Urothelial Cancer

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Background and clinical case study

Marked divergence of muscle-invasive bladder cancer (MIBC) is a well-established and important histopathological feature that has a significant bearing on the diagnosis and clinical outcomes of these cancers[1]. The nested variant of urothelial carcinoma (NVUC) frequently mimics benign lesions of the bladder and, despite a bland cytologic appearance, is often recalcitrant to conventional chemotherapies[2]. We present a NVUC case in which we have piloted a multiparametric precision medicine pipeline - not routinely used to inform the clinical care of patients with MIBC - to guide patient treatment and thus contribute to improving patient outcome.

Patient clinical history:

- 77-year-old male
- Presented with bowel obstruction
- Enhancing plaque-like lesion on bladder wall

- **TURBT**: Solid bladder lesion 45% NVUC
- **Cystoprostatectomy**: – pT3bN2
- Treatment naive

- **2.5 Muts/Mbp**:
  - **Tier II**: Somatic *TERT* & *CCND1* mutations
  - **Tier III**: *FGF3* GAIN, *ATM* & *TP53* LOSS

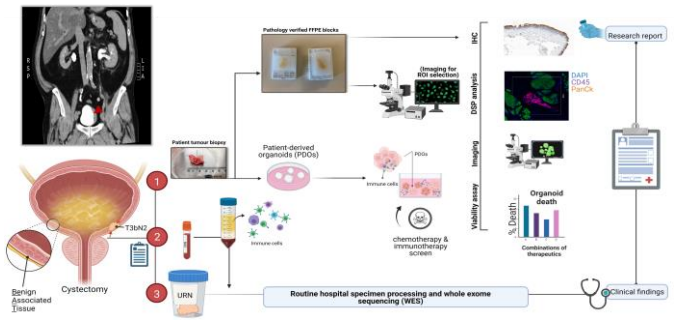

Figure 1. Overview of the experimental plan and approach for 1, 2 and 3, including, immunohistochemistry (IHC) and GeoMx Digital Spatial Profiling (DSP). NMIBC, non-muscle invasive bladder cancer; DSP, digital spatial profiling; BeAT, benign associated tissue.

NVUC is enriched for luminal bladder cancer cells

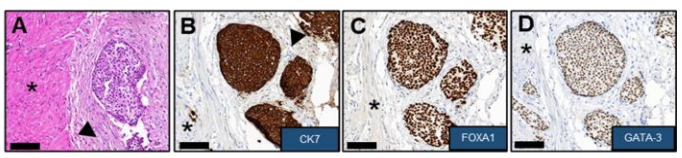

Figure 2. NVUC histological findings. **(A)** Representative images of H&E-stained slide from patient showing variably sized, infiltrative cancer cell nests invading deep into the muscularis propria. Immunohistochemical profile of NVUC showing positive staining for luminal markers **(B)** CK7, **(C)** FOXA1, and **(D)** GATA-3.

NVUC immune infiltrations are enriched for CTLs and have high TIM-3 expression

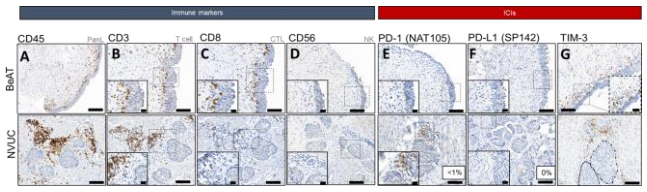

Figure 3. Representative images of immune cell marker staining in NVUC and BeAT. Top row down indicates the adjacent BeAT and NVUC tumour invasive front with the dense infiltration of **(A)** Pan-leucocytes (CD45+), **(B)** T cells (CD3+) and **(C)** cytotoxic T cells (CTLs; CD3+CD8+). Negligible populations of **(D)** natural killer (NK cells; CD56+) observed. **(E)** Positive membranous staining for PD-1 in peritumoral infiltrating cells. **(F)** Negative expression of PD-1. **(G)** Representative IHC staining of high TIM-3 staining in BeAT and adjacent to NVUC invasive regions. Scale bars=100 µm & 50 µm for insets.

CD45 informed region of interest segmentation strategies for 34-plex GeoMX immune cell spatial profiling in NVUC tissue identifies novel immunotherapy targets for patient

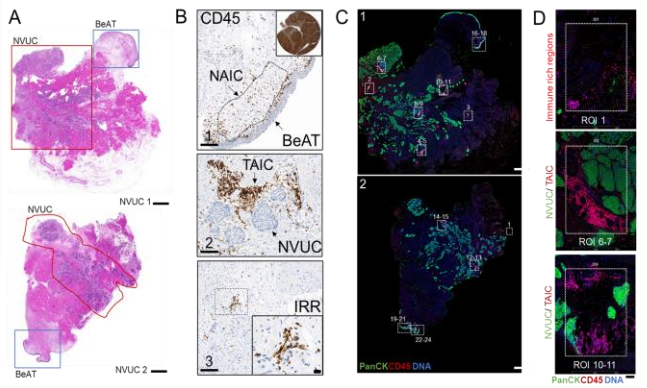

Figure 4. **(A)** Representative H&E images of two regions of NVUC tissue. Red boxes indicate regions of NVUC, while blue indicate BeAT. Scale bar = 5 mm. **(B)** Representative IHC for CD45 regions indicate normal-associated immune cells (NAIC), BeAT, tumour-associated immune cells (TAIC), NVUC & immune rich regions (IRR). **(C)** Regions captured by Nanostring GeoMx Digital Spatial Profiling multiplex proteomic protein assay. **(D)** Spatial profiling was formed on PanCK+ (Tumour) and CD45+ (Immune) regions. Green = PanCK, Red=CD45 and Blue=Nuclei.

NVUC tumour regions are enriched for novel BC targets co-stimulatory molecule OX40L, and checkpoint target B7-H3, when compared to adjacent normal associated urothelial tissue

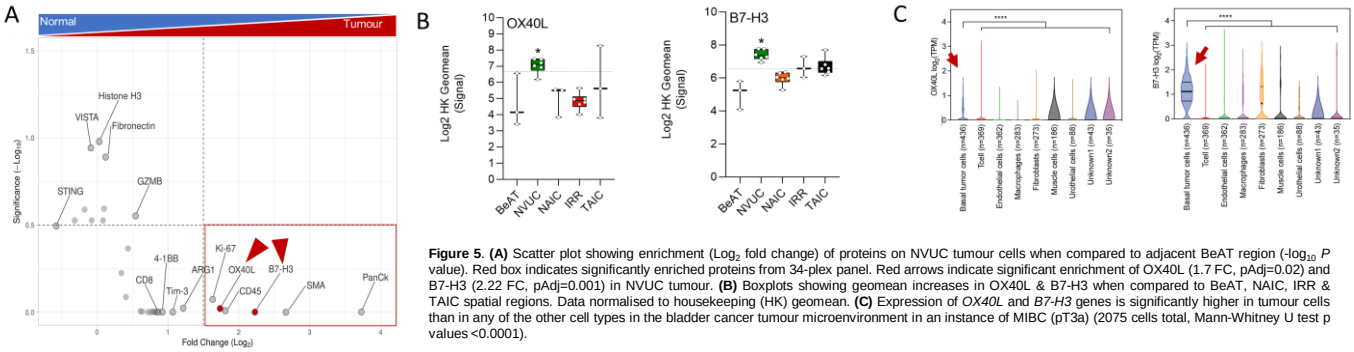

Figure 5. **(A)** Scatter plot showing enrichment (Log_2 fold change) of proteins on NVUC tumour cells when compared to adjacent BeAT region ($-log_{10}$ *P* value). Red box indicates significantly enriched proteins from 34-plex panel. Red arrows indicate significant enrichment of OX40L (1.7 FC, pAdj=0.02) and B7-H3 (2.22 FC, pAdj=0.001) in NVUC tumour. **(B)** Boxplots showing geomean increases in OX40L & B7-H3 when compared to BeAT, NAIC, IRR & TAIC spatial regions. Data normalised to housekeeping (HK) geomean. **(C)** Expression of *OX40L* and *B7-H3* genes is significantly higher in tumour cells than in any of the other cell types in the bladder cancer tumour microenvironment in an instance of MIBC (pT3a) (2075 cells total, Mann-Whitney U test p values <0.0001).

Conclusions

- We redefine a case study incident of a rare nested variant of urothelial carcinoma, including histological features, immunohistochemical markers and mutational landscape.
- The immune profile of the MIBC NVUC tumour microenvironment was defined using spatial profiling
- Notably, co-stimulatory molecule OX40L & ICI B7-H3, are potential immunotherapy targets for this MIBC case

Our pilot study demonstrates that it is feasible to incorporate spatial profiling to wholly investigate novel immune-targeting therapies

Acknowledgements

1. Australian Institute of Health and Welfare, 2019; Available at: https://canceraustralia.gov.au/affected-cancer/cancer-types/bladder-cancer/bladder-cancer-statistics.
2. Venyo, A. *Adv Urol*. 2014: 192720.

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