The Interplay Between the Tumour Microenvironment and Myeloid Cells in Bladder Cancer Immunotherapy Resistance

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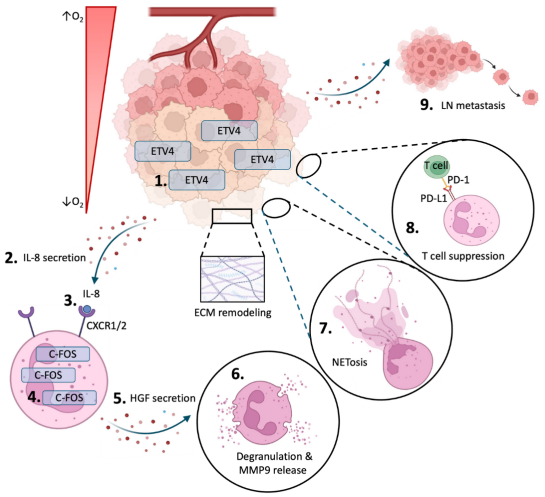
INTRODUCTION

- Bladder cancer (BlCa) is the only top-10 cancer without improved prognosis rates in the last 20 years [1].
- Combining immune checkpoint inhibitors (ICI) with extracellular matrix (ECM)-targeting antibody-drug conjugates has doubled overall survival in muscle-invasive bladder cancer (MIBC) patients compared to chemotherapy alone [2].
- While PD-1/PD-L1 blockade has improved bladder cancer survival rates, **only 20% of patients have a sustained response** [3].
- Hypoxia is a hallmark of solid tumours and a feature of ~70% of solid BlCa tumours [4].
- Neutrophil infiltration is a poor prognostic marker associated with hypoxia in MIBC [5, 6] but, the immunosuppressive pro-tumour mechanisms are unclear.

NEUTROPHILS PROMOTE BLADDER CANCER IN RESPONSE TO HYPOXIA

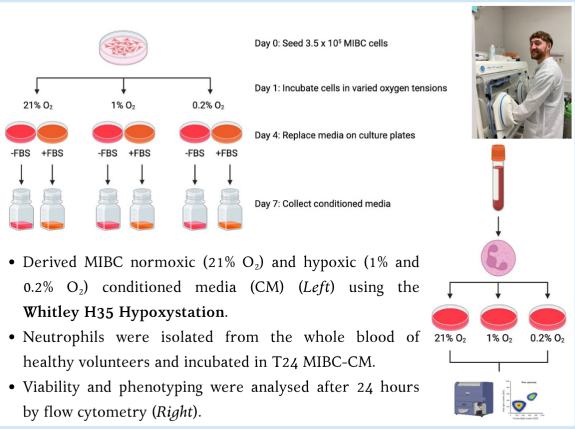


1.) Hypoxic MIBC cells upregulate ETS variant transcription factor 4 (ETV4)
2.) Releasing interleukin-8 (IL-8) into the tumour microenvironment (TME).
3.) IL-8 binds to chemokine receptors 1 and 2 (CXCR1 and CXCR2).
4.) Upregulates c-FOS signalling.
5.) C-FOS induces hepatocyte growth factor (HGF) secretion, exacerbating 2.) IL-8 secretion from MIBC cells and creating a positive feedback mechanism.
6.) Neutrophils degranulate and release matrix metalloprotease 9 (MMP9).
7.) MMPs & NETosis remodel the extracellular matrix (ECM).
8.) ECM remodelling enhances infiltration of PD-L1$^+$ neutrophils which exhaust T cells and dampen ICI responses.
9.) Bladder tumours evade immune targeting and undergo vascular endothelial growth factor A (VEGFA)-induced lymph node metastasis.

EXPERIMENTAL AIMS:

1. Determine whether hypoxia-associated extracellular proteins can induce polarisation of pro-tumour neutrophils.
2. Identify biomarker(s) for patient stratification to determine who will benefit from immunotherapy.
3. Test novel therapeutic strategies that synergise with immunotherapies.

METHODOLOGY



- Derived MIBC normoxic (21% O_2) and hypoxic (1% and 0.2% O_2) conditioned media (CM) (*Left*) using the **Whitley H35 Hypoxystation**.
- Neutrophils were isolated from the whole blood of healthy volunteers and incubated in T24 MIBC-CM.
- Viability and phenotyping were analysed after 24 hours by flow cytometry (*Right*).

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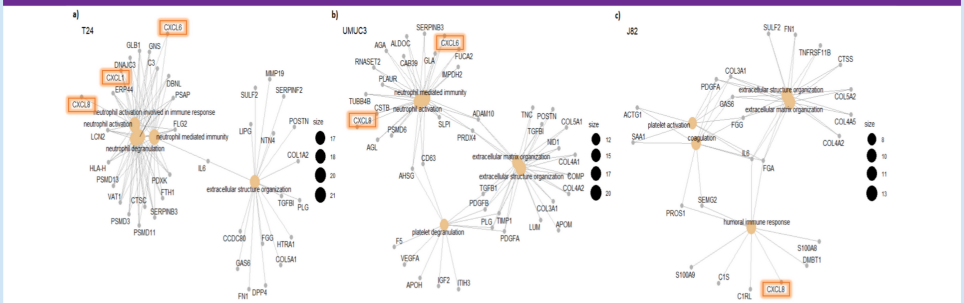
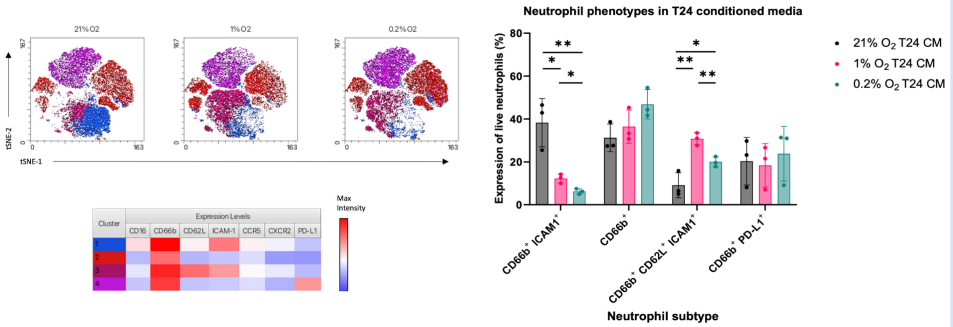
RESULTS

HYPOXIC BLADDER CANCER APPEARS TO ALTER ECM AND NEUTROPHIL FUNCTIONS

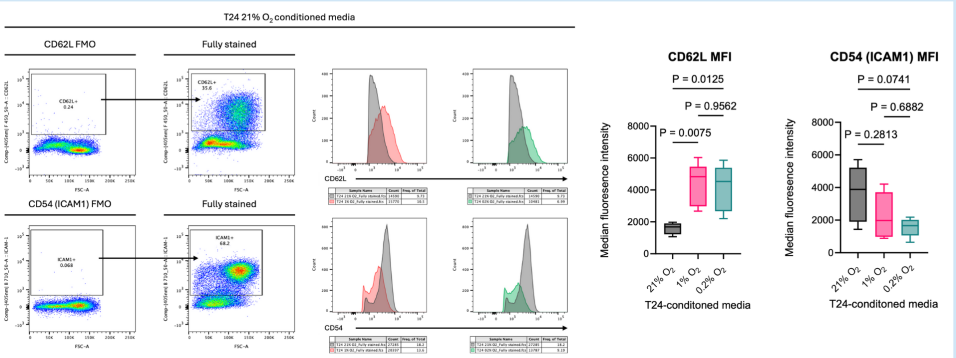


- a) Proteomic profiling of hypoxic (0.2% O_2) T24-CM showed the presence of chemokines: CXCL1, CXCL6 and CXCL8, which are known ligands of the commonly expressed tumour-associated neutrophil receptor CXCR2 [7].
- Pathway enrichment analysis shows these chemokines are associated with neutrophil activation, degranulation, and general neutrophil-mediated immunity. Whereas, IL-6 was associated with factors involved in ECM remodelling processes.

"ANTI-TUMOUR IMMUNE STIMULATORY" NEUTROPHIL SUBTYPE IS ENRICHED IN NORMOXIC (21%O_2) T24-CONDITIONED MEDIA VERSUS HYPOXIC (1% AND 0.2% O_2)

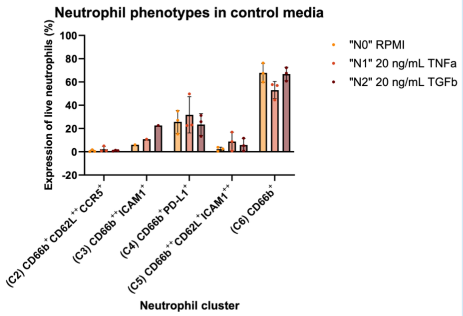


- tSNE and clustering analysis identified four phenotypically distinct neutrophil subtypes following incubation in 21%, 1% and 0.2% O_2 T24-CM (*Left*).
- ICAM1$^+$ "anti-tumour T cell-stimulating" population was significantly enriched in the normoxic CM while the hypoxic CM stimulated more "unprimed/inactive" CD62L$^+$ neutrophils with potential immune suppressive tumour-promoting capacity (*Right*) (n=3).



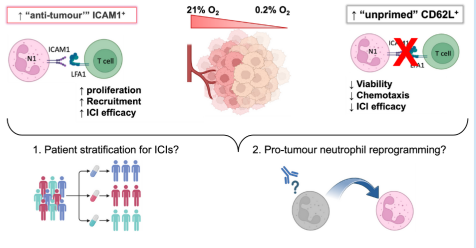
- Representative gating strategy (*Left*), histogram plots (*Centre*) and quantification of CD62L$^+$ and CD54 (ICAM1)$^+$ neutrophils cultured in 21%, 1% and 0.2% O_2 T24-CM (*Right*) (n=5). Statistical values calculated using one-way ANOVA.

TNF-ALPHA DOES NOT STIMULATE THE NEUTROPHILS SEEN IN T24-CM



- tSNE and clustering analysis identified five phenotypically distinct neutrophil clusters following incubation in unstimulated RPMI media, RPMI +20ng/mL TNF-alpha and RPMI +20ng/mL TGF-beta.
- Combination and concentration of cytokines are likely crucial to drive the expression of CD62L and ICAM1 in the four neutrophil subtypes following culture in T24-CM.

SUMMARY, WORKING HYPOTHESIS & FUTURE DIRECTIONS



- ICAM1$^+$ neutrophils stimulate the proliferation and recruitment of T cells to the TME boosting ICI efficacy and tumour suppression [8].
- Reduced infiltration of ICAM1$^+$ subtypes indicates immunosuppression in hypoxic tumours.
- Future work - single-cell RNA sequencing to uncover targetable molecular pathways for anti-tumour reprogramming.
- Chemotaxis and NETosis functional assays to characterise pro- and anti-tumour neutrophils in T24-conditioned media.
- Clinical output - mechanistic insights could be predictive and/or prognostic for solid tumours resistant to ICIs.