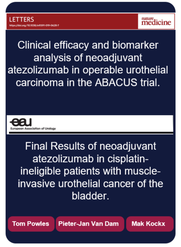
Spatial immune architecture and tumor–T cell proximity are associated with response to neoadjuvant atezolizumab in muscle-invasive bladder cancer.

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CellCarta

BACKGROUND

ABACUS Investigator neoadjuvant phase II trial



Neoadjuvant PD-L1

single-arm phase 2 study investigating two cycles of atezolizumab before cystectomy in 95 patients with muscle-invasive urothelial cancer. 80 patients for biomarker analysis.

Endpoints

Primary: Pathological complete response
Secondary: Safety, relapse-free survival and biomarker analysis.
Supplemental analysis with relapse rates

Samples



Investigations

Pre-existing CD8+ T cell immunity
TGF-β driven Tumor Microenvironment
TMB, DNA damage & DNA alterations
Gene Expression & Protein Biomarkers

Resistance Biomarkers

No association - TMB and PD-L1
Stromal factors - high TGF-β and FAP
T cell exclusion from tumor nests
High ctDNA at any stage of treatment

Response Biomarkers

Pre-existing CD8 T cells
CD39 GZB FOXP3 activation markers
IFNγ and activated T cell gene expression

2 yr DFS 70% versus 40-50% for SoC. Results in primary tumors differ from metastatic.

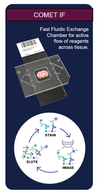
Muscle invasive bladder cancer is a aggressive subset of bladder cancer which occurs in approximately 25% of the 570,000 newly diagnosed cases each year. Standard of care is neoadjuvant cisplatin-based chemotherapy followed by cystectomy but up to 50% of patients are unfit to receive cisplatin-based chemotherapy and undergo upfront cystectmy. These patients have a poor prognosis and novel treatment approaches are needed.

ABACUS was a single-arm phase II investigator study of two rounds of neoadjuvant atezolizumab anti-PD-L1 in muscle-invasive bladder cancer (MIBC) before cystectomy. Clinical response was not associated with tumor mutational burden or PD-L1 expression,Pathologic response,disease-free survival (DFS) and relapse free survival (RFS) were associated with baseline expression of interferon gamma/CD8 effector cell gene signatures and the induction of high levels of activated (GZB+) and tumor-reactive (CD39+) CD8+ cells after treatment.

METHODS

Hyperplex COMET Panel

mIF staining of a subset of ABACUS samples pre and post therapy.



ABACUS Panel

Target	Clone
HLA-ABC	EMR8-5
CD39	EPR20627
CD68	KP1/M7
HLA-DR	TÜK72+
DC/LAMP	M3D36D06
CD11b	10D12.1D
GZMB	EPR1344
LAG3	17B4
CD163	D6U1J
FoxP3	LL263
CD3	2SM4/57
CD4	BLR037F
CD8	C8/144B
CD20	L26
CD56	LUN56
CD11c	BLR415BH
CD68	KP1
PDL1	73-10
αSMA	1A4
PD1	EPR4877(2)
aGFP	BLR037E
panCK	AE1/AE3

ABACUS Phenotypes

Cell Type	Phenotype
Helper T Cell	CD3+/CD4+/-/CD8-
Regulatory T cell	CD3+/CD4+/CD8-/FOXP3+
Cytotoxic T Cell	CD3+/CD4-/CD8+/GZMB+ or GZMK+
Exhaustued T Cell	CD3+/CD8+/PD-1+/LAG3+ CD3+/CD4+/PD-1+/LAG3+
Antigen Exposed T cell	CD39+/CD8+
Dendritic Cell	CD11c+/HLADR+/CD68-
Activated Dendritic Cell	CD11c+/HLADR+/LAMP3+/CD68-
Macrophage	CD11c+/HLADR+/LAMP3+ /CD68+
Tertiary Lymphoid Structure	CD20+/CD3+/HLADR+/LAMP3+
NK Cells	CD56+/GZB+
Epithelial Tumor Cells	PanCK+

Multiplex spatial immunophenotyping of ABACUS tumor specimens.

Formalin-fixed paraffin-embedded tumor samples collected before and after neoadjuvant atezolizumab treatment were analyzed using a 22-marker COMET™ panel. Single-cell segmentation, phenotype assignment, and spatial analyses were performed using the SPARTA computational framework to characterize immune composition, cellular neighborhoods, and cell-cell interactions associated with clinical response.

Image and Data Flow

Expansive Data Set Per Slide at 4 slides per run, fewer cases stained, but extensive insight accuired per staining run.

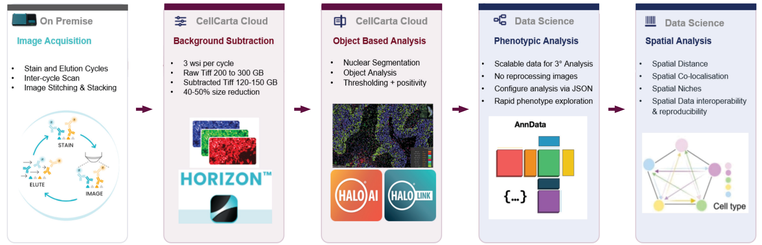


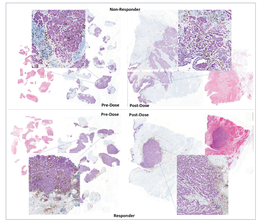
Image Analysis

Raw COMET images were reviewed and quality controlled in Horizon Viewer. Background subtraction was performed using the isotype control cycle rather than the autofluorescence cycle to maximize background signal removal. Background-corrected images were imported into HALO AI v3.6 for analysis. Nuclei were segmented using the embedded pre-trained AI IF nuclear segmentation module. Cell classification was then performed using dynamic thresholding, with target- and sample-specific positivity thresholds applied to optimize classification accuracy and minimize false-positive and false-negative detections.

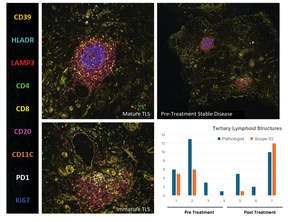
Phenotype and Spatial Analysis

Spatial data analysis was performed using the CellCarta SPARTA framework. Every cell in the tissue was assigned a phenotype using a custom JSON configuration that encodes the phenotype definitions for consistent and reproducible cell annotation across samples. The distance of each cell to the nearest cell of every other phenotype was calculated using the scimap package which performs a quantitative measure of how closely different cell populations are positioned relative to one another. Spatial colocalization between each pair of phenotypes was quantified as an interaction score, computed with high-performance code developed in-house in Rust and capturing the degree to which phenotype pairs are found in close spatial association. Immune triads, defined as a dendritic cell in close contact with both a CD4+ T cell and a CD8+ T cell within a 25 µm radius, were identified using custom CellCarta algorithms.

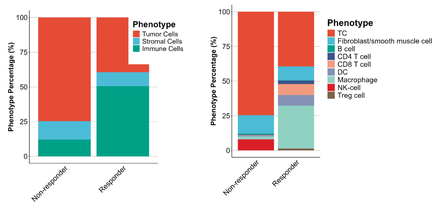
RESULTS



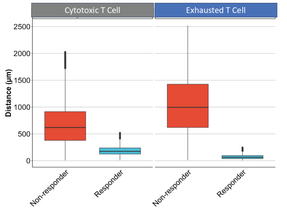
Inflammation comprised of CD8+ T cells is present in tumour nests and tumour associated stroma pre and post neoadjuvant therapy in both responders and non-responders. H&E stained tissue and PanCK (purple) -CD8 (brown) stained FFPE tissue pre and post two rounds of atezolizumab neoadjuvant therapy. Pre-existing CD8 T cell infiltration is a pre-requisite but not the only requirement for response to neoadjuvant therapy.



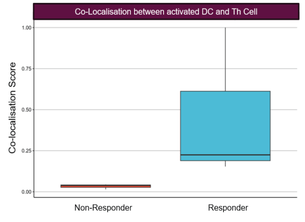
Immature and mature Tertiary Lymphoid Structures (TLS) present pre and post anti-PD-L1 treatment. TLS were scored by a pathologist using H&E and COMET stained slides. Clusters of >50 infiltrating mononuclear cells were identified the H&E stained slide and the COMET stained slide was used to confirm the phenotype of clustered cells. H&E wsi were analysed using Lunit SCOPE IO. This AI powered algorithm segments regions considered TLS within the tumor microenvironment and quantifies their area and distribution. TLS counts obtained using Lunit SCOPE IO showed strong correlation with pathologist enumeration (Pearson r=0.84), but demonstrated a negative bias, with Lunit SCOPE IO identifying on average 2.3 fewer TLS per sample than manual assessment. Given the relatively small 7 case set used for this comparison, the agreement obtained was considered good.



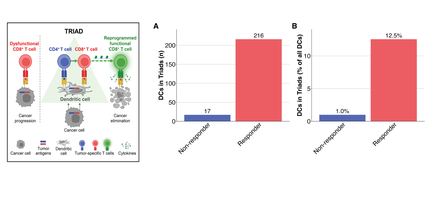
Immune Landscape Differs Between Responders and Non-Responders. Stacked bar plots show the relative abundance of major tissue compartments (left) and immune cell phenotypes (right). Responding tumours demonstrate increased immune infiltration, with immune cells comprising approximately 50% of the tissue compared with ~12% in non-responders. This immune-rich microenvironment is characterized by increased macrophage, dendritic cell, CD4 T-cell, and CD8 T-cell populations, consistent with a more inflamed tumour phenotype. In contrast, non-responding tumours are dominated by tumour cells and exhibit a greater proportion of NK cells with reduced overall immune infiltration.



Spatial proximity analysis of immune cell populations. Boxplots show the distribution of cell-to-cell distances (µm) for cytotoxic T cells and exhausted T cells in responder and non-responder samples. Responders showed substantially shorter distances than non-responders, consistent with increased spatial interaction and immune niche formation involving activated cytotoxic and exhausted T-cell populations.



Activated dendritic cell-T helper cell interactions are enriched in responders. Co-localisation scores derived from permutation-based spatial analysis demonstrate increased proximity between activated DCs and Th cells in responder tumours. The formation of these cellular interaction niches may support antigen presentation, T-cell priming, and sustained IFNγ-associated immune activation linked to therapeutic response.



Spatially organized dendritic cell-T cell interaction niches are associated with clinical response. Immune triads are defined as a dendritic cell in close proximity to both a CD4 and CD8 T cell. Triads were enriched in responding tumours and increased following atezolizumab therapy. These multicellular structures are thought to support local antigen presentation, CD4 T-cell activation and cytokine production, and activation of cytotoxic CD8 T-cell responses. Results suggest that effective anti-tumour immunity in ABACUS is associated with the formation of coordinated immune interaction hubs.

CONCLUSIONS

The CellCarta SPARTA spatial workflow enabled integrated characterization of tumor, immune, and stromal populations within the ABACUS cohort.
Clinical response to atezolizumab is associated with a more inflamed and immunologically diverse tumor microenvironment.
Activated dendritic cell-CD4 T-cell interactions are increased in responding tumours. Productive APC-CD4 T-cell interactions are a defining feature of response to neoadjuvant PD-L1 blockade.
Immune triads and TLS support the formation of functional anti-tumour immune niches. Response is associated with formation of higher-order immune cellular networks rather than simple increases in immune-cell abundance alone.
Functional T-cell states are spatially organized within the tumor microenvironment and may reflect ongoing immune activation in responding patients. Cytotoxic and exhausted T cells demonstrate closer spatial proximity in responders.
Spatial immune architecture provides information beyond immune cell density alone and may represent a novel predictive biomarker for neoadjuvant checkpoint blockade in MIBC.

KEY TAKEAWAYS

Response to neoadjuvant atezolizumab is associated with the development of spatially organized immune ecosystems, characterized by coordinated dendritic cell-T cell interactions, immune triad formation, tertiary lymphoid structures, and concentrated cytotoxic T-cell niches. These spatial features may represent clinically relevant biomarkers of effective anti-tumour immunity in muscle-invasive bladder cancer.

Send inquiries to isales@cellcarta.com with SITC 2026 Spatial Immune Architecture in email subject.

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