Abstracts

1465 MULTIPLEX IMAGING IDENTIFIES UNIQUE IMMUNOPHENOTYPIC AND SPATIAL CHARACTERISTICS ASSOCIATED WITH RESPONSE TO IMMUNE CHECKPOINT INHIBITORS (ICIS) IN METASTATIC UROTHELIAL CANCER (MUC)

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Background ICIs increase survival in mUC but only a subset (~15–25%) of patients experience durable disease control. Differences in the tumor microenvironment (TME) might underlie such differential responses. However, the complex network of cellular interactions within the TME that associate with response and resistance to ICIs remains underexplored.

Methods Multiplex Immunohistochemical Consecutive Staining on a Single Slide (MICSSS)1 was performed on UC specimens (N=40) from CheckMate 2742 prior to treatment with nivolumab. 9 immunohistochemical stains (PD-L1, CD8, CD3, pan-cytokeratin, fibronectin, CD68, FAP, DC-LAMP3, CD11b) were sequentially performed on a single slide per patient. Image processing, whole slide annotation (median 464,534 cells per patient), and intra- and extra-tumoral compartment training were performed using QuPath (figure 1).6 Responders (CR, PR) and non-responders (SD, PD) were defined per REGIST v1.1. Immunophenotypic designations of ‘inflamed’, ‘excluded’, and ‘desert’ were defined via tumor margin CD8 analysis.7 Lymphoid aggregates were identified morphologically with dense CD3 positivity. Single-cell spatial analysis was performed defining neighborhoods as the 25 nearest neighboring cells.

Results TME characterization demonstrated inter-tumoral heterogeneity, both in the intra- and extra-tumoral compartments (figure 2). Responders contained 2–3 fold increased intra-tumoral CD8 cells, though no cell types were significantly altered in comparison to non-responders. In contrast, extra-tumoral CD3, CD8, CD3CD8+, DC-LAMP (PD-L1+ and PD-L1+), and PD-L1+ CD11b cells were significantly enriched in responders (figure 3). Inflamed tumors were more prevalent and excluded/desert tumors less prevalent in responders, with inflamed tumors containing increased intra-tumoral T cell and DC-LAMP infiltration. There were no significant differences in infiltrate composition between inflamed responders and inflamed non-responders, while excluded/desert responders demonstrated enrichment for extra-tumoral DC-LAMP cells (PD-L1+ and PD-L1+) and intra-tumoral PD-L1- DC-LAMP cells as compared to excluded/desert non-responders (figure 4). Responder tumors also contained an increased density of lymphoid aggregates, which were found in closer proximity to tumor regions, were associated with increased survival, and were comprised of a greater degree of DC-LAMP cells (figure 5). Spatial analysis of extra-tumoral cells identified a unique immune and tumor-enriched PD-L1+ neighborhood (cluster 0) predominant in responders, and a distinct CD11b and tumor-based neighborhood devoid of PD-L1 and other immune cells (cluster 2) predominant in non-responders (figure 6).

Conclusions Multiplex immunohistochemistry identified unique immunophenotypic and spatial TME features specific to mUC responders to ICI. Both increased infiltration and the geographic arrangement of T cells, dendritic cells, and PD-L1 positivity, particularly in the extra-tumoral compartment, may prove key in identifying responders to ICI.

Trial Registration This is a secondary translational analysis from NCT02387996 (CheckMate 275, CA209–275)

REFERENCES


Ethics Approval The initial clinical trial NCT02387996 obtained appropriate ethics approval, and was conducted in accordance with Good Clinical Practice guidelines defined by the International Conference on Harmonisation. All participants provided informed consent before taking part in the study based on the principles of the Declaration of Helsinki. Approval was granted from local institutional review boards or ethics committees at each center (as published).

Abstract 1465 Figure 1 MICSSS analysis pipeline. 9 sequential immunohistochemical stains were performed on a single slide to each patient. Images were then co-registered to the single cell level, confirmed at each quadrant of the specimen. Tissue annotation and cell segmentation were performed on whole tissue specimens (median 464,534, range 134,796–1,255,791 cells per patient). Stain positivity was confirmed at multiple regions for each stain and patient. Intra- and extra-tumoral compartment training was performed on multiple regions.
Abstract 1465 Figure 2 Whole cohort TME characterization. (A) Waterfall plot demonstrating the TME composition breakdown from each cell type, each column represents an individual patient (B) Waterfall plots (top), scatter plots (bottom) demonstrating the percentage of each cell type within the intra-tumoral (left) or extra-tumoral (right) compartments.

Abstract 1465 Figure 3 Compartment-specific TME infiltrate in responders and non-responders. Comparison of lymphoid, myeloid (including PD-L1 positive and negative subsets), and matrisome cell types between responders (blue) and non-responders (gray) in the (A) intra-tumoral and (B) extra-tumoral compartments. Bar plots (right) represent the ratio of the median cell concentration between responders and non-responders, dotted lines indicating >2-fold difference. *P<0.05, **P<0.01, ***P<0.001

Abstract 1465 Figure 4 TME immunophenotypes in responders and non-responders. (A) Example images of inflamed, excluded, and desert TMEs, CD8 in pink, CK in brown (left) Breakdown of immunophenotype designation by CD8 analysis at the tumor outer margin and tumor inner margin (center). Breakdown of immunophenotype designation in responders and non-responders (right). Bar plots represent the ratio of the median cell concentration between (B) inflamed and desert/excluded patients, (C) inflamed responders and inflamed non-responders, and (D) excluded/desert responders and excluded/desert non-responders (right). Dotted lines indicating >2-fold difference. *P<0.05, **P<0.01, ***P<0.001, P>0.05 and <0.1 listed.

Abstract 1465 Figure 5 Lymphoid aggregate analysis in responders and non-responders. (A) Density of lymphoid aggregates per patient. (B) Distance from each lymphoid aggregate to the nearest tumor region. Black bar represents the median (left). Distribution of distances (right). (C) Kaplan-Meier curve for overall survival of patients separated by median density of lymphoid aggregates. (D) Bar plot represents the ratio of the median cell concentration within lymphoid aggregates between responders and non-responders, dotted line indicates >2-fold difference. *P<0.05, **P<0.01, ***P<0.001, ***P<0.0001

Abstract 1465 Figure 6 Neighborhood Analysis of Extra-Tumoral Tissue. (A) Neighborhood characterization was completed by applying K-Nearest Neighbors (k = 25) to single-cell expression data, where the mean expression value over 25 nearest neighbors (including center cell) was averaged over each stain to create a mean expression vector for each neighborhood. UMAP dimensionality reduction was applied on neighborhoods, where the centre cell is classified as within the extratumoral tissue, to visualize distribution of neighborhoods along with corresponding antibody expressions. (B) K-means clustering was applied on neighborhood expression vectors (n-clusters = 4) and is visualized in UMAP space. (C) Composition of each sample by clusters was conducted and stratified between responders/non-responders by each cluster (**P<0.01). (D) Cluster characterization visualized via clustermap where each row represents a cluster and columns represent average expression of given marker for each cluster (z-scores shown, red = high expression, blue = low expression).

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