

Spatial phenotypes in metastatic prostate cancer associate with PSA response and early remodeling during [¹⁷⁷Lu]Lu-PSMA-617 plus pembrolizumab

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Background: Despite PSMA-PET selection, clinical responses to [¹⁷⁷Lu]Lu-PSMA-617 remain heterogeneous, and tissue features associated with response remain incompletely defined. We applied spatially resolved multiplexed proteomics to determine epithelial and tumor-microenvironmental states associated with PSA response and early remodeling in longitudinal tissue from PRINCE, a phase 1b/2 study of [¹⁷⁷Lu]Lu-PSMA-617 plus pembrolizumab in metastatic castration-resistant prostate cancer (mCRPC).

Methods: Metastatic biopsy specimens obtained from patients enrolled in the PRINCE clinical trial were profiled by imaging mass cytometry (IMC) using a 47-channel custom antibody panel to capture diverse prostate epithelial, stromal, and immune cell states. Pixel-level expression values were aggregated and analyzed at a single-cell level of resolution using the 'steinbock' analysis pipeline. Single-cell data were integrated across experimental runs using the Harmony batch-correction approach prior to defining cell states (via graph-based cell clustering) and performing cell-state enrichment analyses. Associations between pre-treatment cell states and best observed PSA percentage decline after combination [¹⁷⁷Lu]Lu-PSMA-617 plus pembrolizumab were evaluated in pretreatment "screening" biopsies (n=18); PSA50 and PSA90 endpoints were also assessed. Differences between cell populations observed in metastatic tumors sampled before and after Cycle 1 of treatment were evaluated.

Results: Thirty participants with tissue samples available were included: 25 archival localized-tumor specimens, 18 pretreatment "screening" mCRPC tumor biopsies, 13 mCRPC biopsies sampled after Cycle 1 of treatment, and 4 mCRPC biopsies sampled upon confirmed clinical disease progression after initial therapy. IMC profiling yielded 713,781 cells representing 25 phenotypic cell states (i.e. clusters). In the tumor-microenvironment compartment, amongst screening biopsies, a stromal cell state (cluster C5) was associated with less favorable PSA decline (Spearman rho=0.618, p=0.0063). This cell state overexpressed bone-specific proteins in the absence of pan-Cytokeratin, CD45, and PSMA, supporting an osteo-lineage rather than epithelial or immune cell subtype. Fourteen of 18 screening biopsies were sampled from bone, and cells comprising cluster C5 were observed near-exclusively in bone specimens. Moreover, spatial analysis revealed that these C5 cells localized away from PSMA-high epithelial cells and near other vimentin-expressing cells, orthogonally supporting C5 as a non-epithelial cell subtype of the tumor microenvironment. In the epithelial compartment, we observed that greater median PSMA expression in the epithelial compartment was associated with deeper PSA decline (rho=-0.496, p=0.036). Longitudinal analysis of paired pretreatment and post-Cycle 1 mCRPC biopsies revealed that PSMA-high epithelial cell fraction decreased in 11/12 participants (median 19.0% to 5.2%).

Conclusions: Baseline epithelial and tumor microenvironmental states were associated with PSA response to [¹⁷⁷Lu]Lu-PSMA-617 plus pembrolizumab, and tissue remodeling is observable early during treatment. These exploratory findings suggest that both epithelial and tumor microenvironmental tissue features may be pertinent biomarkers to increase specificity of treatment selection for radiopharmaceutical therapy.

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