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Using multiplex *in situ* hybridization and machine learning to evaluate bi-specific binder targets in metastatic Castration Resistant Prostate Cancer (mCRPC)

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Biomarkers such as DNA, RNA and protein are powerful tools for understanding basic cellular functions, diagnosing clinical diseases and identifying optimal therapies. In particular, RNA is an ideal indicator of the dynamic gene expression changes that occur in an organism. RNAscope® *in situ* hybridization assay, a novel technology established by Advanced Cell Diagnostics (California, USA), was utilized to generate RNA co-expression data to assist with prioritization of multi-antigen tumor targeting pairs. We developed a platform that allows binder pairs for a prostate-specific membrane antigen target to be evaluated for co-localization in tumor cells using fully automated multiplex *in situ* hybridization with immunohistochemistry on FFPE tissue. In conjunction with image acquisition and data analysis, imaging algorithms were developed to generate quantitative data for binder pairs co-localization, spatial distribution and proximity measurements within tumor and stromal architecture.

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