Quanticell™, a novel histopathology technology for visualization and quantitation of membrane PSMA demonstrated in cell lines and clinical tissue

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Background

^{68}Ga-PSMA PET is a highly promising modality for staging prostate cancer due to its higher detection rate compared to conventional imaging techniques. Both PET/CT and PET/MRI combined with PSMA radiotracers are able to detect tumors with very high levels of PSMA expression. However, PSMA-PET lacks the sensitivity to distinguish low versus high expression. Radioconjugates, such as Lutetium-177 (^{177}Lu)-PSMA-617, and PSMA-BiTE® molecules (AMG 212, AMG 160) have been used to detect PSMA-positive disease. PSMA-PET and tissue-based immunoassays have been used to evaluate PSMA expression in clinical tissues, however, standard immunohistochemistry and immunofluorescence assays are not sensitive enough to detect low PSMA expression in prostate cancer biopsies. Lack of sensitivity may prevent proper response predictions and could deny anti-PSMA treatment to qualified patients that harbor low PSMA expression. Here, we present a new histopathology assay, Quanticell, to enable the sensitive and specific detection of PSMA in prostate tumors.

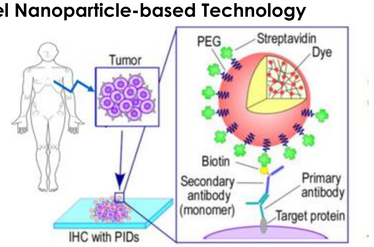
Objective

These studies were designed to evaluate Quanticell, a new assay based on novel phosphor-integrated dots (PID) detection technology on PSMA-expressing cell lines and clinical tissue. The Quanticell technology was compared to standard immunohistochemistry for:

- Specificity
- Sensitivity
- Dynamic Range
- Quantification

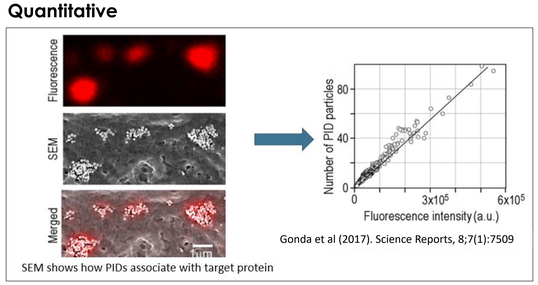
Methods

Novel Nanoparticle-based Technology



Quanticell Schematic. Quanticell consists of highly fluorescent, extremely uniform streptavidin-coated nanoparticles. Quanticell technology involves a standard immunostaining workflow using commercially available primary antibodies and biotinylated secondary antibodies.

Quantitative



Quanticell Quantitation. The number of PID particles per cell based on fluorescence microcopy and electron microscopy were plotted against the respective fluorescence intensity. A dedicated analysis software was developed to quantitate the number of particles in microscopy images.

Specificity

Specific Signal Only in PSMA-Expressing Cell Lines

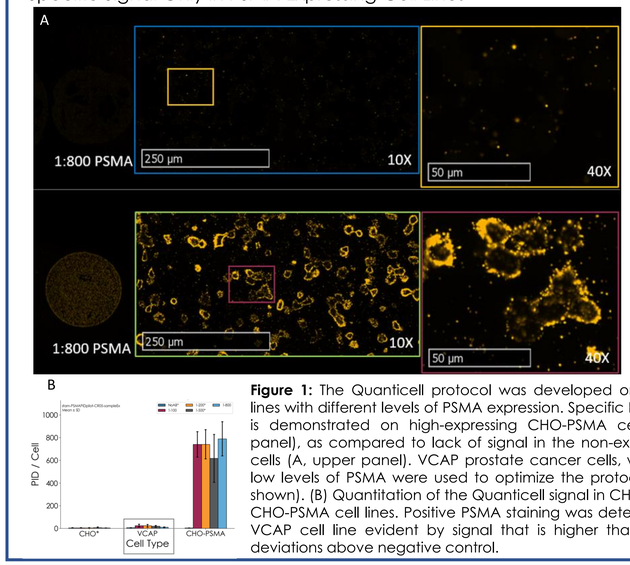


Figure 1: The Quanticell protocol was developed on several cell lines with different levels of PSMA expression. Specific PSMA staining is demonstrated on high-expressing CHO-PSMA cells (A, lower panel), as compared to lack of signal in the non-expressing CHO cells (A, upper panel). VCAP prostate cancer cells, which express low levels of PSMA were used to optimize the protocol (data not shown). (B) Quantitation of the Quanticell signal in CHO, VCAP and CHO-PSMA cell lines. Positive PSMA staining was determined in the VCAP cell line evident by signal that is higher than 3 standard deviations above negative control.

Sensitivity

Comparison Between Quanticell Detection and DAB Detection Using Comparable Analysis Workflow

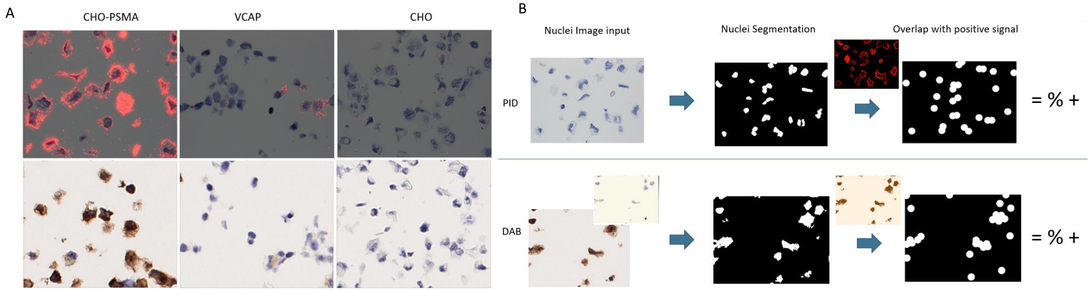
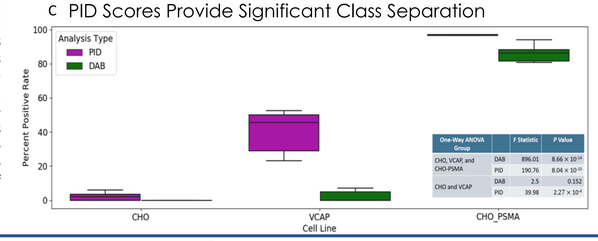


Figure 2: The sensitivity of the Quanticell assay was compared to chromogenic diaminobenzidine (DAB) for detection of PSMA. (A) Cells were stained by the Quanticell method (upper panel) and for DAB (lower panel). We developed comparable analysis workflows to quantitate the percent PSMA-positive cells identified by each technique. In both detection methods, we used hematoxylin to identify and count the number of cells. Fluorescent Quanticell signal or chromogenic DAB signal were then overlayed on the hematoxylin image to determine % cells that are PSMA-positive (B). The Quanticell assay identified PSMA-positive VCAP cells with significant separation from the PSMA-negative CHO cells, demonstrating the improved sensitivity of Quanticell in detecting low PSMA expression levels.

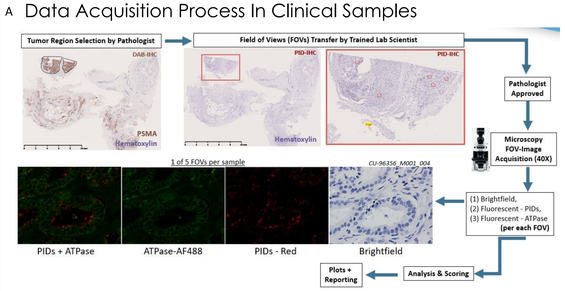
C PID Scores Provide Significant Class Separation



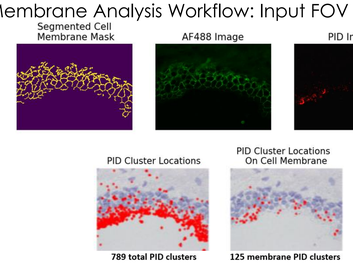
One-Way ANOVA Group		F Statistic	P Value
CHO, VCAP, and CHO-PSMA	DAB	896.01	8.66×10^{-15}
	PID	190.76	8.04×10^{-10}
CHO and VCAP	DAB	2.5	0.152
	PID	39.98	2.27×10^{-5}

Quantification and Dynamic Range

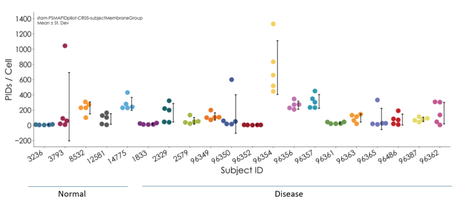
A Data Acquisition Process In Clinical Samples



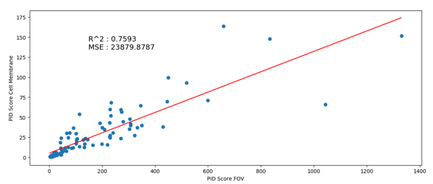
B Membrane Analysis Workflow: Input FOV Image



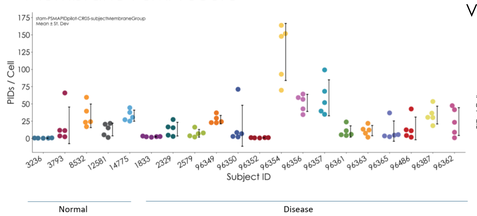
C Total PSMA Score



D Correlation Between Total and Membrane PSMA



Membrane PSMA Score



E Variance in Quanticell Signal Across Clinical Samples

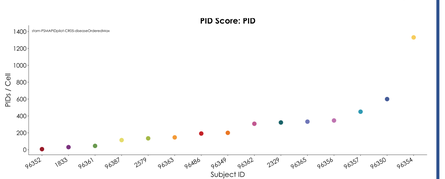


Figure 3: (A) From the whole slide image of stained clinical samples, high magnification fluorescence microscopy was used to acquire multiple fields of view (FOV) across the regions containing tumor cells. The image acquisition was performed in three channels: PSMA staining by PID (red channel), sodium potassium ATPase to mark the cell membrane (Alexa Fluor 488), and hematoxylin staining to mark the cell nuclei (brightfield image). (B) To enable analysis of membrane-associated PSMA, the cell membrane was segmented and a mask of the membrane was applied to the Quanticell or PID channel. (C) Using an established imaging and image analysis workflow, a proprietary algorithm for analysis was developed and conducted to score total PSMA expression (upper plot) and membrane-specific PSMA expression (lower plot). Analysis was conducted on biopsies from healthy subjects (n=5) and prostate cancer subjects (n=15). The PSMA signal (PID/cell) was determined per FOV, represented by each data point, with 5 FOVs analyzed per subject. (D) High correlation was observed between the total PSMA score and the membrane-PSMA score. (E) From the total PSMA analysis, the highest FOV scores per subject across all 20 subjects were plotted to demonstrate the variance between subjects and the dynamic range of Quanticell detection method.

Summary

The Quanticell technology provides a sensitive, specific method to quantitate target expression levels and can be applied for evaluation of human tumor samples

Specificity

- Anti-PSMA PID staining shows high specificity of detection of PSMA

Sensitivity

- An optimal signal to noise ratio was achieved with a high PSMA-specific signal and minimal background staining
- PID provides increased sensitivity in detecting low PSMA-expressing cells, as compared to standard IHC

Quantification

- The Quanticell assay is suitable for quantitation of PSMA levels per cell

Dynamic Range

- The Quanticell assay has the potential to evaluate PSMA expression across a broad range of expression levels

Disclosures

This study was funded by Amgen, Inc.
JB is employed by and holds stock in Amgen Inc.
JG was employed by and held stock in Amgen Inc. at the time the work was done.