Optimization of an ultrasensitive, quantitative immunoassay for detection of CD20 in Non-Hodgkin's Lymphoma (NHL) FFPE samples

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Abstract # 41

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Background

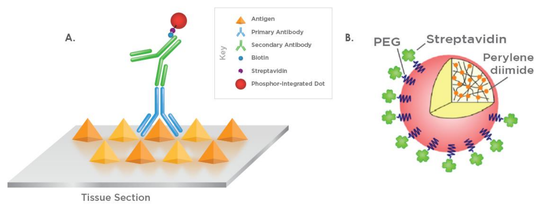
CD20 is a membrane B cell marker expressed from the pre-B cell stage until terminal differentiation to plasma cells. CD20 is found on the majority of mature B-cell neoplasms, including diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma. Importantly, it is the target of the monoclonal antibody, rituximab as well as autologous T cell and bispecific, immune-directing antibody therapies (BiTE®) in clinical development. It has become increasingly appreciated that one mechanism of resistance to rituximab-containing therapies is downregulation of CD20 [1, 2]. Histologically, differences in CD20 density are difficult to determine by standard chromogenic IHC methods. Development of an assay that provides highly sensitive and accurate detection of CD20 levels in the tissue context may help to assess whether there is a minimum CD20 threshold associated with response to rituximab or other CD20-targeted therapies.

Objective(s)

- Develop and benchmark a novel Quanticell™ assay for sensitive and quantitative detection of CD20 expression that outperforms DAB-based immunohistochemistry.
- Apply CD19 biomarker co-expression and a digital pathology-based approach for quantitative analysis of CD20 expression in FFPE biopsy samples from NHL patients.
- Assess CD20 Quanticell assay performance for detection sensitivity and accuracy relative to an established digital spatial profiling technology.

Quanticell Technology

Novel Nanoparticle-based Technology



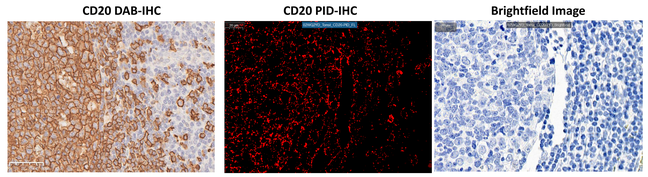
Overview of Quanticell technology. (A) The standard antibody-based workflow relies on an indirect detection method, whereby a primary antibody is added followed by a biotinylated secondary antibody to detect target antigens in tissue. The signal is detected using streptavidin-coated fluorescent PID nanoparticles. **(B)** PIDs are stable and highly fluorescent particles that have ~2,400 streptavidin molecules linked via polyethylene glycol (PEG) chains on its surface to provide significantly higher reactivity with secondary antibodies [3,4].

Image Analysis

- PID visualization and quantitation is done using a standard optical system and dedicated image-processing software.
- The expression levels of target proteins in tissues is reported as the average PID number/cell or the average PID number/unit area (PID score/100 µm^2).

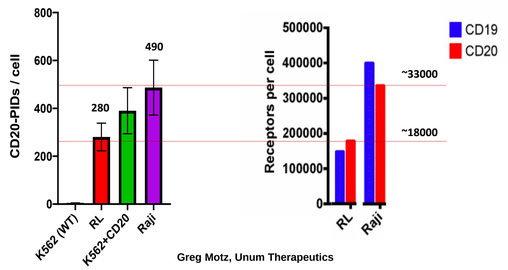
Assay Development

Step 1: Developing a CD20 Quanticell Assay



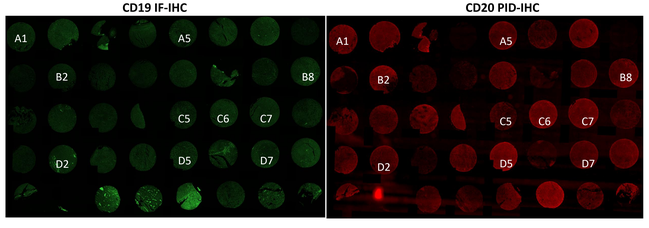
- DAB-IHC on tonsil tissue was utilized to compare staining pattern of different commercially available CD20 antibodies (Abcam, SP32 and L26 clones).
- SP32 clone was selected for CD20 Quanticell assay development.
- CD19 IF-IHC + CD20 Quanticell IHC was developed to ensure only B cell-rich biopsy regions are selected for CD20 Quanticell analysis.

Step 2: Benchmarking the CD20 Quanticell Assay against Flow Cytometry



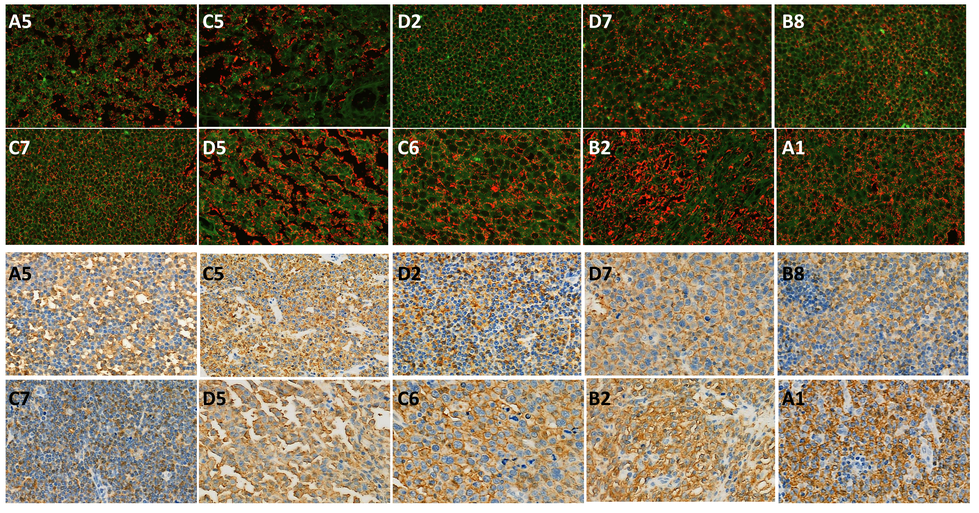
- CD20 expressing cell lines were used to benchmark assay performance.
- RL and Raji cell lines showed 1.75-fold and 1.83-fold increase in CD20 expression by Quanticell and flow cytometry assays, respectively.

Step 3: Assay Transfer on a FFPE B Cell Lymphoma Tumor Array

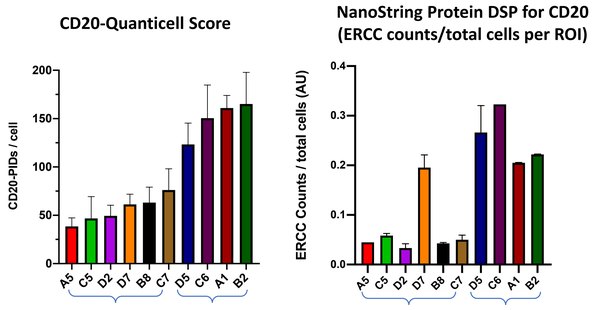


- A commercially available B lymphoma TMA (n=39 cores, LN08/TMA-1702) was stained for CD19 IF-IHC and CD20 PID-IHC.
- Ten different NHL biopsies (CD19+) were selected for CD20 analysis by traditional semi-quantitative IHC, Quanticell assay, and NanoString protein DSP.

Quanticell Assay Performance on Clinical Tissue



- Human tonsil tissue was used to confirm CD20 assay performance as a Quanticell singleplex or duplexed with CD19 IF-IHC.
- To assess sensitivity of CD20 detection, a non-B cell core (CD19 and CD20 negative by IHC) was analyzed.



- NanoString Protein Digital Spatial Profiling (DSP) enables quantitative evaluation of approximately 60+ different protein targets within specified regions of interest on FFPE tissue sections, including evaluation of CD20.
- Both the Quanticell CD20 and NanoString Protein DSP detection for CD20 appropriately cluster similar cores together into CD20^{low} and CD20^{high} categories.
- While NanoString DSP for CD20 displays enhanced sensitivity compared to IHC, Quanticell demonstrated the ability to measure CD20 expression more sensitively over a broader dynamic range when compared to the DSP method.

Notes:
- NanoString values for CD20^{low} cores appear at or near the limit of detection, limiting resolution at the low end of CD20 expression.
- Core D7 is an outlier from the overall trend, but the exact cause remains unclear.

Summary and Applications

- The novel CD20 Quanticell immunohistochemistry-based assay provides significantly enhanced detection and quantification of CD20 in FFPE tissue samples. This technology may be useful to assess whether there are critical antigen densities associated with response to CD20-targeting therapies.
- This study demonstrates that Quanticell assays are uniquely suited for identifying low expressing biomarkers and can be multiplexed to understand other useful readouts the immune contexture of the tumor microenvironment. Quanticell technology has immediate potential as a translational research tool, particularly in the development of cancer immunotherapies and antibody-drug conjugates.
- The Quanticell technology can be deployed in a CAP/CLIA environment and support exploratory endpoint testing in clinical trials.

References

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Disclosures

This study was funded by Unum Therapeutics. MR and AR were employed by and held stock in Unum Therapeutics at the time the work was done.