Development of a Clinical Trial Immunohistochemistry (IHC) Assay Using a Novel Antibody to CD38

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Introduction

CD38 is a type II transmembrane glycoprotein expressed normally in lymphoid and myeloid cells along with some non-hematopoietic tissue. CD38 is also expressed in many hematologic malignancies. A number of CD38 antibody therapies have been developed, or are under development, that induce cytotoxicity against CD38-expressing cells through multiple pathways. Three drug candidates are currently in clinical testing (daratumumab, isatuximab, and MOR202) while a number of others are in preclinical development. Daratumumab is the only human monoclonal CD38 antibody that is FDA-approved for its anti-tumor activity (multiple myeloma, previously treated). Recent studies have also indicated an immunomodulatory role of anti-CD38 therapies leading to combination immunotherapy trials for various solid tumors (Van de Donk NWCJ).

With the increasing utility of CD38 as a therapeutic target, the potential for CD38 as a predictive biomarker has emerged, thus creating the need for a reliable diagnostic assay. The CD38 IHC DAK-CD38 prototype assay uses the Autostainer Link 48 automated staining platform with integrated DakoLink software. Epitope mapping revealed that the novel DAK-CD38 antibody recognizes a linear epitope. This antibody has the potential to be adapted to Dako Omnis staining platform and additional technologies such as flow cytometry.

Methods

This IHC assay is based on EnVision FLEX visualization technology using CD38 primary antibody, clone DAK-CD38, that has been developed and manufactured by Agilent Technologies. The assay staining protocol was developed for Dako PT Link and Autostainer Link 48 automated IHC platform (Figure 1). Following incubation with the CD38 antibody or the negative control reagent, specimens are incubated with a ready-to-use visualization reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added diaminobenzidine chromogen results in precipitation of a visible reaction product localized to the antigen. Stained slides are then interpreted using a light microscope.

Development of the CD38 IHC DAK-CD38 assay was performed on three non-Hodgkin lymphoma sub-types: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), and mantle cell lymphoma (MCL). For determination of CD38 positive staining, partial and/or complete linear circumferential membrane staining of neoplastic lymphocytes was included. Normal hematopoietic and necrotic cells were excluded from scoring. The total percent positive viable neoplastic lymphocytes with 1+, 2+, and 3+ intensity membrane staining was recorded.

Figure 1. Autostainer Link 48 solution.

Results

Staining:
The CD38 IHC DAK-CD38 assay provides crisp and clear plasma membrane staining with rare, minimal non-specific staining. Figure 2 shows examples of CD38 IHC DAK-CD38 assay staining in DLBCL, FL, and MCL tissues with a range of intensities (0 – 3+). Figure 3 illustrates CD38 staining of three different intensities in a DLBCL specimen.

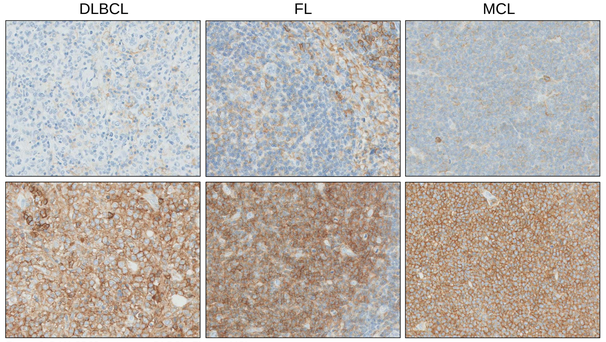

Figure 2. DAK-CD38 staining in DLBCL, FL, and MCL.

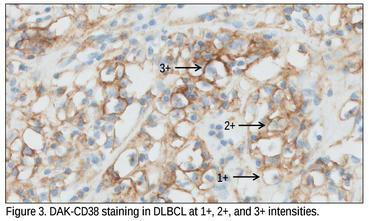

Figure 3. DAK-CD38 staining in DLBCL at 1+, 2+, and 3+ intensities.

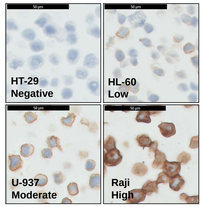

Figure 4. Sensitivity of DAK-CD38 using cell lines.

Sensitivity:
The assay detects the CD38 protein in non-Hodgkin's lymphoma tissues and cancer cell lines.

Cell lines: 14 FFPE cell lines evaluated with the CD38 IHC assay resulted in expected staining from scientific literature. Figure 4 shows images of four cell lines with 0, 1+, 2+, and 3+ intensity CD38 expression.

Results Continued

Tumor tissue: A full range of CD38 expression from 0 % to 100 % positive was observed in 75 DLBCL, 151 FL, and 44 MCL FFPE specimens (Figure 5).

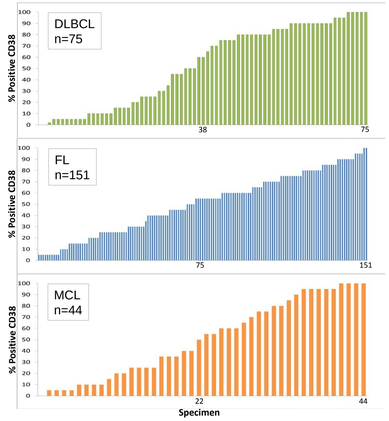

Figure 5. Expression plots of % CD38 positivity in DLBLC, FL, and MCL specimens.

Specificity: The assay demonstrates analytical specificity to CD38 using FFPE specimens other than DLBCL, FL, and MCL.

Tumor tissue: 114 different tumor tissues evaluated with the CD38 IHC assay resulted in expected staining from scientific literature. 11 tumor tissues expressed CD38 (Table 1) and 103 tumor tissues did not express CD38 (not shown).

Table 1. Specificity of DAK-CD38 using various tumor tissues.

Tissue Type	Tumor Type	# expressing CD38 / total tested
Brain/cerebrum	Astrocytoma	4/4
Gallbladder	Adenocarcinoma	1/6
Head & Neck Tongue	Squamous cell carcinoma	1/2
Liver	Hepatocellular carcinoma	1/5
Lung	Adenocarcinoma	1/5
Lymph node	Anaplastic large cell lymphoma	1/1
Ovary	Serous adenocarcinoma	1/1
Prostate	Adenocarcinoma	4/5
Testis	Seminoma	2/2
Uterus	Adenocarcinoma	1/3
Abdominal Wall	Clear cell sarcoma	1/1

Normal human tissue: 30 different normal tissues evaluated with the CD38 IHC assay resulted in expected staining from scientific literature (data not shown).

Results Continued

Western blot: cell lysates with known CD38 expression along with cells transfected with CD38 overexpressing vector (OEL), or an empty vector control, were analyzed by Western blot. A ~45 kDa band for CD38 was observed for cells known to express CD38 and was absent for cells known not to express CD38.

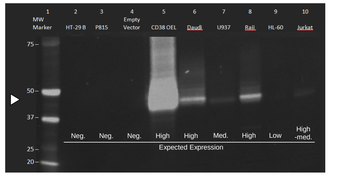

Figure 6. Western Blot of cell lysates using DAK-CD38 antibody. 15 µg of protein loaded per lane.

Precision: The assay produces consistent results in normal day-to-day testing.

For inter-observer precision, average positive (APA) and average negative (ANA) agreement was calculated based on a ≥50 % CD38 expression cut-off (Table 2).

Table 2. Inter-observer agreement results with the 95 % confidence intervals in parentheses.

Agreement	DLBCL (n= 30)	FL (n= 30)	MCL (n= 30)
ANA	93.5 % (86.0-100 %)	95.5 % (89.4-100 %)	95.1 % (88.6-100 %)
APA	93.3 % (85.4-100 %)	95.7 % (88.4-100 %)	95.9 % (89.1-100 %)

Repeatability and within laboratory precision tested intra-run, inter-day, inter-instrument, inter-operator, and inter-lot variables and resulted in acceptable staining variability.

Robustness: The assay produces consistent results across small variations in key assay parameters.

Studies included Target Retrieval Solution (TRS) Temperature, TRS Time, TRS pH, TRS Re-use, Post-TRS Rinse Time, Reagent Volume, Chromogen Volume, Tissue Thickness, Water Source, Run Size, Overnight Run. All robustness tests resulted in acceptable staining variability.

Conclusion

The CD38 IHC DAK-CD38 prototype assay has demonstrated acceptable sensitivity, specificity, precision, and robustness for use in detecting CD38 in DLBCL, FL, and MCL tissues.

References & Disclaimers

Van de Donk NWCJ, Janmaat ML, Mutis T, et al. Monoclonal antibodies targeting CD38 in hematological malignancies and beyond. Immunological Reviews. 2016;270(1):95-112. doi:10.1111/imr.12389.

References pertaining to expected CD38 expression are available on the digital copy of this poster via QR code download.

Some tissue samples used in this study were provided by IUSCC Cancer Center at Indiana School of Medicine for the use of the Tissue Procurement and Distribution Core, which provided Dako North America service, and by the Cooperative Human Tissue Network, which is funded by the National Cancer Institute. Other investigators may have received specimens from the same subjects.

Additional References

Development of a Clinical Trial Immunohistochemistry (IHC) Assay Using a Novel Antibody to CD38
Abstract #7561

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