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Redefining IHC Assays for Powerful, Quantitative Patient Stratification and Companion Diagnostics

INSIDE:

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Elevating IHC Assay Development for Patient Stratification: A New Approach

The use of immunohistochemistry (IHC) for assessing the progression and treatment of cancer has become a staple in oncology research. IHC assays, which enable the observation of processes in the context of tissue, are relatively easy to use and cost effective, making them a valuable tool utilized widely across the industry. On the clinical side, pathologists use IHC staining to diagnose cancer as benign or malignant, determine the stage and grade of a tumor, and identify the cell type and origin of a metastases to identify the primary tumor site. IHC also plays an important role in drug development, where it is used to test drug efficacy by detecting the activity of disease targets.

As the industry works toward personalized medicine, however, higher expectations are being placed on IHC. Tissue is the gold standard in cancer research and diagnosis, and there is thus a significant need for IHC to act as a robust assay for tissue samples for the identification of biomarkers and to support patient stratification and diagnostics. This is a critical step on the road to personalized medicine. Unfortunately, IHC assays as they currently stand were not actually designed to meet this need.

While IHC assays can be very useful in some areas of oncology, they can be less sensitive quantitatively than immunoassays such as ELISA, which is considered a strong assay for liquid samples, and are commonly criticized for not being standardized or consistent. These issues prevent IHC from being able to consistently and effectively help researchers identify biomarkers and create assays to stratify patients, and IHC is thus not always viewed as a robust diagnostic. In fact, despite the high use of IHC assays over the last several decades, the industry has seen relatively little success in validating them as companion diagnostics for cancer drugs – to date, only a handful have been approved by FDA, primarily for breast cancer.[1]

Despite the challenges and industry perception of IHC's capabilities, quantitative, fit-for-purpose IHC assays are in fact possible. With the introduction of automated image analysis, which enables researchers to gather significantly more data from tissue samples, quantitative IHC (qIHC) can become a reliable methodology for identifying biomarkers and stratifying patients. By changing the way we look at, develop and utilize IHC assays, ultimately this tool can improve clinical programs for pharmaceutical companies by facilitating more successful clinical trials and the development of companion / in vitro diagnostics (IVD) and targeted therapies.

IHC for patient stratification is arguably the most important application for pharmaceutical companies and oncology currently, since effective use in this arena directly impacts drug trials and the development of critical companion diagnostics.

[1] U.S. Food and Drug Administration. List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools). FDA Website. 2014. Available at: http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm. Accessed April 6, 2015.

Challenges with Traditional (Qualitative) IHC Assays

Immunohistochemistry is the process of detecting antigens or proteins in tissue cells by leveraging the principal of antibodies binding specifically to antigens. IHC makes it possible to visualize the distribution and localization of specific cellular components within cells and in the proper tissue context.[2]

IHC staining is used in a number of ways, from early stage research to understand the distribution and localization of biomarkers, to drug development, and to the diagnosis of abnormal, cancerous cells in the clinical setting.[3] IHC for patient stratification is arguably the most important application for pharmaceutical companies and oncology currently, since effective use in this arena directly impacts drug trials and the development of critical companion diagnostics.

Despite the ubiquitous use of IHC assays, several inherent challenges exist:

- **Tissue Collection & Handling**: Preparation of the tissue sample, including proper collection, fixation and sectioning, is critical to end results of an IHC assay. If tissue collection and handling protocols are not standardized or properly followed, clinical trial results can be negatively impacted. One small step, such as a trial site not transporting a tissue sample to the reference lab for staining within the time period stated in the protocol, may result in drastically different results that can alter the direction of a trial. Unfortunately, avoidance of deviation from the protocol in standard hospital practice is not always a priority without education as to its importance, and it's not always possible for pharmaceutical companies to ensure adherence to protocols by clinical trial sites.
- **Antibody Behavior**: Beyond tissue collection and preparation issues, there are challenges with the way IHC assays are designed. IHC staining is based on antibody behavior and the pathologist's visual inspection of the components within the tissue, which may not always be precise. Additionally, antibodies may not have the sensitivity required for the test to be successful. Antibodies may partially or weakly bind to sites on nonspecific proteins (reactive sites) that are similar to the cognate binding sites on the target antigen; a great amount of non-specific binding causes high background staining which will mask the detection of the target antigen.[4] There are methods researchers can use to reduce background staining, but with IHC design relying on antibody behavior and pathologist visualization, there can still be a high level of inaccuracy.

In theory, a morphology-based assay such as IHC should be a highly effective means to define the expression of a target molecule of interest, especially if the target is a protein[5]; and in reality, researchers need to be able to rely on IHC to effectively identify biomarkers in order to stratify patient populations. However, this is an expectation that IHC can't currently meet, and over the last decade IHC as a platform for biomarkers has been challenged by more quantitative molecular assays with reference standards – but that lack important morphologic context.[6] For example, the HER2 assay, the only FDA-approved predictive IHC biomarker, should have a straightforward evaluation with a positive HER2 result being defined as 3+ IHC staining. However, its reproducibility is disconcertingly low and 20% of HER2 assays performed in the field were incorrect when the same specimen was reevaluated in a central laboratory.[7] As we seek to identify specific patient sub-populations for drug trials, measurements need to be much more precise; and in order for IHC to become a top tier biomarker assay, it must provide truly quantitative data on par with non-morphologic assays.[8]

2 Duraiyan J, Govindarajan R, Kaliyappan K, Palanisamy M. Applications of Immunohistochemistry. Journal of Pharmacy & Bioallied Sciences. 2012 Aug; 4 (Suppl 2): S307-S309.
3 Ibid.
4 Ramos-Vera JA, Miller MA. When tissue antigens and antibodies get along: revisiting the technical aspects of immunohistochemistry--the red, brown, and blue technique. Veterinary Pathology. 2013; 51: 42-87.
5 Dunstan R, Wharton K, Quigley C, Lowe A. The Use of Immunohistochemistry for Biomarker Assessment—Can It Compete with Other Technologies? Toxicologic Pathology. 2011; 39 no. 6: 988-1002.
6 Ibid.
7 Ibid.
8 Ibid.

It's clear that IHC assays need to be re-evaluated and reconceived in order to meet the patient stratification needs of personalized medicine. Automated image analysis, which enables researchers to view all tissue features in context, provides an opportunity to innovate IHC. It allows researchers to unlock information which cannot be conceived of with traditional IHC, and thereby create robust, reliable and powerful assays to meet important patient stratification goals. In short, advanced image analysis can enable researchers to use IHC to its full capacity.

Unlocking the Value of IHC Assays Through Automated Image Analysis

In order for IHC to become truly effective, it needs to be standardized, reliable and quantitative. This requires digitization of images and the use of image analysis, and for IHC pathology to become a quantitative versus descriptive discipline.[9] According to a study in Toxicologic Pathology, "Without digitization there can be no accurate quantitation; without quantitation, there can be no standardization; and without standardization, the value of morphology-based IHC assays will not be realized."[10]

Many of the challenges with IHC assays stem from the simple fact that IHC has been commonly used in a non-standardized and non-regulated way over time. Fortunately, these issues can be addressed. IHC assays can in fact go from being qualitative to quantitative and become a reliable methodology and diagnostic test. With the use of automated image analysis, qIHC can be held to the same standards as ELISA or western blot but with the added value of tissue context.

Uncovering the specific solutions for making IHC a robust diagnostic requires a more in-depth understanding of the issues that currently prevent it from doing so. The two primary challenges with IHC center around its inability to identify clear cutpoints for patient stratification and the lack of a standard curve. These issues can be effectively addressed with the use of automated imaging technology.

Identifying Cutpoints

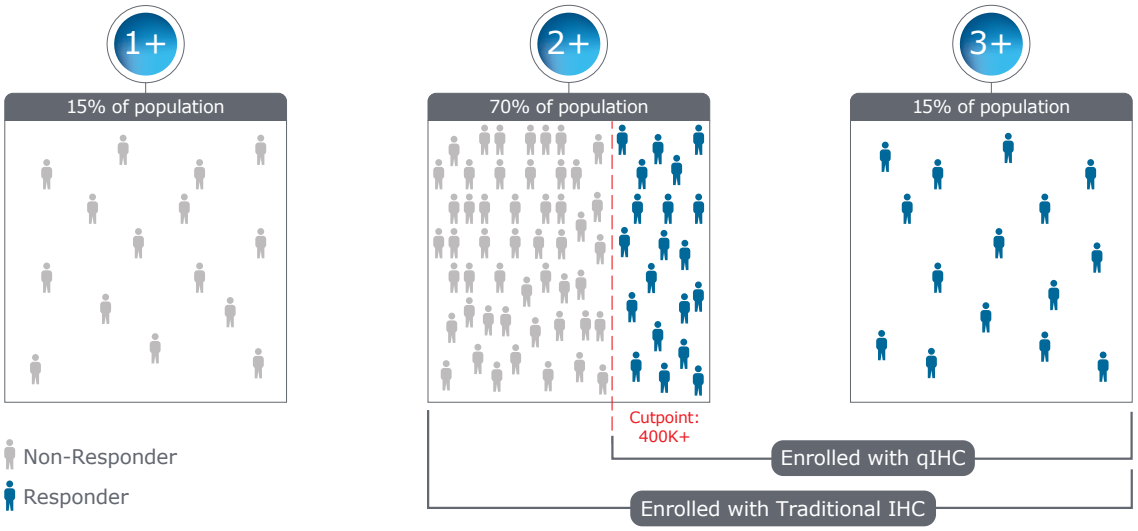
Inaccurate identification of cutpoints is a significant issue – and in fact at the very crux of the problem – in terms of where qualitative IHC assays are lacking. Simply put, if a cutpoint can't be found, the assay can't be used to stratify patients.

Traditionally, IHC assays are designed by optimizing the tissue staining visually: the cutpoint is determined based on the pathologist's visual review of the expression levels identified by the antibody and the distinctions he or she can make between them. If the antibody performs well and binds to a high expression protein, that's where the cutpoint will be drawn, or "binned", since it's what the pathologist can see. This is an arbitrary line, however, since it is based on the antibody's behavior versus a specific measurement or variable with biological relevance.

If the only reliable antibody for staining IHC does not select for expression levels that are significant mechanistically or from a biological standpoint, a pathologist could miss entire expression levels important to stratifying patients, as illustrated in the following scenarios.

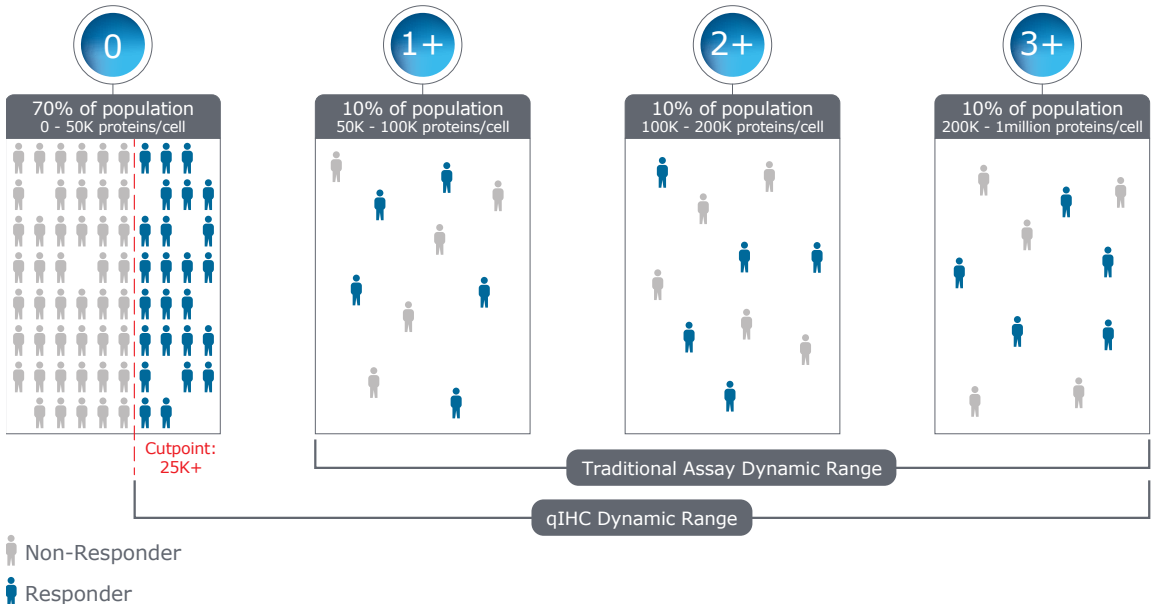
9 Ibid.
10 Ibid.

SCENARIO 1:
Lack of appropriate scoring for IHC assay = Too many non-responders in clinical trial



In this common scenario, an IHC assay uses an antibody that detects 50,000 – >1 million proteins/cell. Pathologists can visually bin patients into categories of 50,000-100,000 proteins/cell, 100,000-500,000 proteins/cell, and 500,000->1million proteins/cell. Using this standard scoring method, the clinical protocol calls for patients in the 2+ and 3+ population to be enrolled, with the 2+ population making up 70% of the patients. However, the biologically relevant cutpoint is actually at ≥400,000 proteins/cell. Therefore, enrollment of the full 2+ population leads to trial results that do not demonstrate medical value in the 2+ population due to two-thirds of the population not responding. This ultimately results in either no further development of the drug or 20% of the population not receiving a valuable treatment. However, the use of quantitative IHC with image analysis would have shown the biologically relevant cutpoint to be at ≥400,000 proteins/cell; thus, if the clinical protocol enrolled patients using qIHC methodology, and thereby including only responders, the trial would have demonstrated response to the drug in the 2+ population due to the correct population of patients having been in the trial.

SCENARIO 2:
Lack of IHC assay sensitivity = Inability to select the correct patients for trial



In this scenario, an IHC assay uses an antibody that detects 50,000 – 1 million proteins/cell. Pathologists can visually bin patients into categories of 50,000-100,000 proteins/cell, 100,000-200,000 proteins/cell, and 200,000-1 million proteins/cell. The clinical trial is enrolling allcomers and using a traditional IHC assay to bin patients for biomarker expression. Surprisingly, the people who respond are negative for the biomarker, and had this group been excluded in a prospective study, the limited number of patients and responders potentially would have limited the drug's clinical advancement or an entire larger population of responders would have missed out on treatment. Using quantitative IHC methods, however, it is determined that the dynamic range of the traditional IHC assay is not sensitive enough to identify the correct biologically relevant cutpoint of the biomarker, which is at 25,000 proteins/cell. By developing the assay with qIHC methods, the correct population would be identified and enrolled in the trial.

Cutpoint identification based on antibody behavior and pathologist visualization is not precise enough for patient stratification in the current research environment. Automated image analysis, however, enables pathologists to find a cutpoint based on measured variables that are biologically relevant. It becomes a quantitative rather than a qualitative decision. Pathologists are able to collect all the useful and relevant data from a tissue sample (thus maximizing the information they get from each sample), view it in context, and select a cutpoint based on measured features or other variables that may be missed by visual observation alone, rather than relying solely on the antibody's behavior.

Ultimately this means that through image analysis, pathologists can identify patient stratification lines that previously could not be found. This approach can lead to significant advancements in personalized medicine goals.

Creating a Standard Curve

All robust assays in the industry have a standard curve and their limitations are thus understood and accounted for in assay design. Traditional IHC assays, however, lack a standard curve, which prevents researchers from being able to test the assay repeatedly over time and from knowing what variables – such as non-standardized tissue collection practices, as mentioned above – will impact the test results. It also prevents them from knowing the dynamic range of the assay, such as in the example above; if the antibody detects a minimum of 50,000 receptors but the cutpoint is at 25,000, researchers might think their "negative" patients are responding to the drug.

With image analysis, however, a standard curve can be implemented and assay development can be standardized. There are three main areas a standard curve can improve IHC assays:

Validation Precision and Accuracy

Understanding the performance of the assay is critical to clinical trial research. Validation of the assay allows researchers to understand the limitations of their test so they know when to trust it and what variables may change the results. Since qualitative IHC assays lack a standard curve, they are validated through the review of three independent pathologists and pathologist confirmation. This is of course an important step, but is not accurate or precise enough for a personalized medicine setting.

Image analysis allows researchers to develop and follow important protocols, conduct more robust validation and understand the limitations of the assay – whether they be the expression range or variations in collection practices – ultimately enabling them to conduct better research and trust their test results. With a standard curve developed through image analysis, researchers can:

- **Develop stronger tests based on their knowledge of how the assay reacts to variables.** With image analysis, researchers can design the test based on how the assay reacts in standard practice, and understand how variables – such as if a hospital doesn't follow tissue collection protocols – will impact the outcomes.

- **Implement better protocols for sample collection and handling.** This is important since, as noted above, lack of standardization with tissue collection can create enormous issues since some proteins are heavily impacted by variations in collection processes. It's critical for the pharmaceutical company to know the test's requirements so it can write clinical trial protocols that will ensure it gets the information it needs. If, for example, the pharmaceutical company knows that a time lapse in tissue staining will negatively impact test results, it can incorporate a time requirement into the protocol; it can also spend more time educating its clinical sites and emphasizing the importance of that protocol. There is no way to know ahead of time if timing – or any other variable – is an issue unless that variable is tested during assay development and then standardized, which is not possible without image analysis.

- **Capture and save important data for repeated testing and comparison, maximizing tissue samples that have been gathered.** With a standard curve, researchers know the expression range and how the assay performs within that range since it can be tested repeatedly. Image analysis also allows comparisons across multiple cohorts of studies. This is in contrast to traditional staining where a sample is scored, but the performance cannot be compared to other cohorts – the sample data exists in isolation and therefore other conclusions cannot be drawn.

Monitoring & Measurement of Test Variability

Measurement of test variability refers not only to variations in collection and handling of tissue, but also variations between antibody lots. Researchers must be able to test antibody variations with each batch, but the changes are often subtle enough that the pathologist can't detect them through visualization alone. Without a standard curve, this data cannot be effectively compared.

Image analysis and the implementation of a standard curve enables pathologists to measure and compare changes over time in the assay, so they can make more informed decisions about the drug's performance overall and among specific patient populations. This is crucial to patient stratification and developing companion diagnostics.

Reproducibility

The lack of a standard curve also creates issues with reproducibility. If a test cannot be reproduced, it cannot be considered accurate and thus will not become a robust diagnostic. A standard curve developed with automated image analysis provides proof that the assay is running within the range of the requirements. Reproduction is no longer an issue: the test can be run hundreds of times and the pathologist can be confident that it will be the same each time, or at the minimum have the measure of the degree of variability that exists to inform the personalized of their scoring.

Practical Application of Image Analysis in Quantitative IHC Assay Development

Applying automated image analysis early in the process of IHC assay development can lead to significantly better results and a better test. When an image analysis solution that can analyze tissue features in context is implemented during the design phase, and a standard curve is created, qIHC can become a true fit-for-purpose assay. As outlined above, if pathologists know the requirements ahead of time, they can make qIHC assays what they need them to be from the beginning – thus saving time and frustration and improving outcomes. Similarly, if a pharmaceutical company knows the test requirements at the start, it can write and implement stricter protocols and prevent inaccurate test results from impacting clinical trials. The use of image analysis supports pharmaceutical companies in creating more standardized tests and ensuring their clinical trial sites are aware of and complying with key changes to standard protocols.

In essence, by utilizing a tool that can gather all the relevant data needed, in context, to effectively stratify patients, and which provides the ability to create robust, precise and reproducible assays, pharmaceutical companies can more effectively move drugs and companion diagnostics through the pipeline and onto the market.

Example from the Field: Developing Rigorous IHC Assays for Companion Diagnostics

The application of automated image analysis in qIHC has already moved beyond discussions of what is conceptually possible to demonstrating tangible benefits in the research setting. One top pharmaceutical company recently implemented image analysis with IHC to identify candidate companion diagnostics across its oncology pipeline, and obtained significant results.

Challenge

Trying to predict response to treatment with its monoclonal antibodies blocking a target family of receptors, the company wished to establish IHC assays to determine the receptor numbers on a cell-by-cell basis. This would allow the company to comprehensively and consistently assess tumor heterogeneity and identify subgroups of patients that may differ in their response to treatment. To achieve this, a mechanistically relevant scoring method for a novel IHC assay had to be determined, and the limited dynamic range of the chromogenic IHC assay had to be expanded.

Approach

The company used a standard curve array to standardize the assay and the clinical samples used for quantitation. Immunoflourescent IHC was employed to increase the ability to quantitate the limited dynamic range of the assay, and image analysis was employed to quantitate images.

Results

With the use of image analysis, a clinically relevant scoring modality was determined that would not have been detectable by traditional chromogenic staining and pathology scoring. This new scoring modality was translated into a chromogenic IHC assay optimized for pathologist scoring around the clinically relevant cutpoint. This resulted in significant improvement in retrospectively identifying more qualified patients for treatment.

The results of this study illustrate how better assays can result from approaching IHC assay development and IHC quantification with advanced image analysis. The level of standardization and validation achieved was significant for the rigorous qIHC assays needed in companion diagnostics development.

Conclusion

As personalized medicine becomes a requirement for the future of cancer care, improving patient stratification in order to determine the best population for treatment is becoming critical. qIHC assays have the ability to enhance patient stratification and the development of companion diagnostics, but with the current standard approach – where assays are tied to how the antibody behaves and what the pathologist can visualize – relevant data goes uncollected and ultimately the industry misses out on drugs that have a high potential to go to market.

The use of qIHC assays for improved patient stratification can be a reality; with image analysis, which enables researchers to gather significantly more data and analyze tissue features in context, pathologists gain the support and better information they need to make critical decisions. Image analysis enables researchers to make qIHC assays more certain, uncover and analyze data that could not previously be seen, and effectively stratify patients. Ultimately this means improved clinical research programs and the ability to develop and move more drugs through the pipeline for the advancement of cancer care.

For more information on how contextual image analysis can be incorporated into qIHC assay development, please contact Definiens.

Image analysis enables researchers to make qIHC assays more certain, uncover and analyze data that could not previously be seen, and effectively stratify patients.

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