

Introduction

Integrin Beta-6 (IB6) is overexpressed in multiple solid tumor types and is a proposed negative prognostic indicator in many cancers, including NSCLC.^{1,2} IB6 combines exclusively with the α v integrin subunit to form the α v β 6 heterodimer, which is frequently upregulated in epithelial cancers and promotes tumor progression.³

An investigational antibody-drug conjugate (ADC), sigvotatug vedotin (SV), is currently being studied in non-small cell lung cancer (NSCLC) and is directed to the novel target IB6.

Integrin Beta-6 IHC 6.2A1 pharmDx (Dako Omnis) is an investigational immunohistochemistry assay developed for the detection of IB6 protein expression and is being used to assess IB6 expression in the ongoing SigVie-002 (NCT06012435) trial. Recent data showed this assay is sensitive, specific, and precise in detecting IB6 protein expression in a large set of non-squamous NSCLC (nsqNSCLC) specimens.⁴

Tumor heterogeneity, originating from genomic instability, is a hallmark of cancer.⁵ Here we present data on IB6 biomarker heterogeneity and further elaborate on the assay's reproducibility as it relates to other clinically relevant assay parameters.

Methods

This assay uses the Monoclonal Mouse Anti-Human Integrin Beta-6, Clone 6.2A1 on the Dako Omnis staining platform. IB6 expression in nsqNSCLC was evaluated by calculating the percentage of viable invasive tumor cells showing partial or complete IB6 membrane and/or cytoplasmic staining at $\geq 2+$ staining intensity. The clinical diagnostic status of IB6 High or IB6 Low was determined using the cutoff $\geq 50\%$.

Tumor heterogeneity was assessed by comparing staining across 200 μ m of a formalin-fixed, paraffin-embedded (FFPE) block (Intra-Block), between blocks from the same tumor (Intra-Case), and between blocks from primary and metastatic tumor sites in the same patient (Primary vs. Metastatic). Other clinically relevant assay parameters were also tested, including tissue section thickness across 3 – 5 μ m, glass slide vs. digital whole slide image (WSI) scoring using the Philips UFS scanner, and preanalytical variables such as cold ischemia and fixation times.

Statistical analysis included calculation of overall agreement (OA), which was performed by summing the number of concordances within each block when compared to the consensus diagnostic. For some studies, negative percent agreement (NPA) and positive percent agreement (PPA) were also calculated.

Results

Intra-Block

Intra-Block testing resulted in 96.3% OA (n=54).

Table 1. Intra-Block results summary.

Specimen Distribution Summary			
Definition	% $\geq 2+$ Range	# Blocks	% Blocks
Negative	<50	28/54	51.9%
Positive	≥ 50	26/54	48.1%
Total Near Cutoff	40-60	18/54	33.3%
All Pair-Wise Comparisons Summary			
Concordant Negatives	Concordant Positives	Discordances	Total
1	26	2	54
Agreement Summary			
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)	
		Lower-bound: 2.5%	Upper-bound: 97.5%
OA	96.3%	90.7%	100.0%

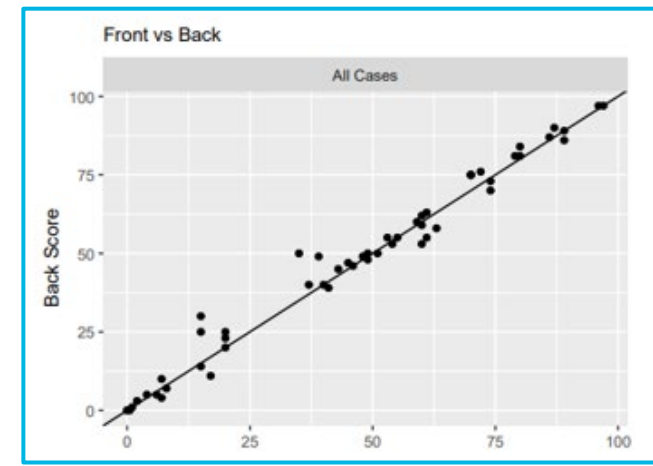


Figure 1. Intra-Block results plot. Front section IB6 score is plotted on the x-axis, and back section IB6 score is plotted on the y-axis.

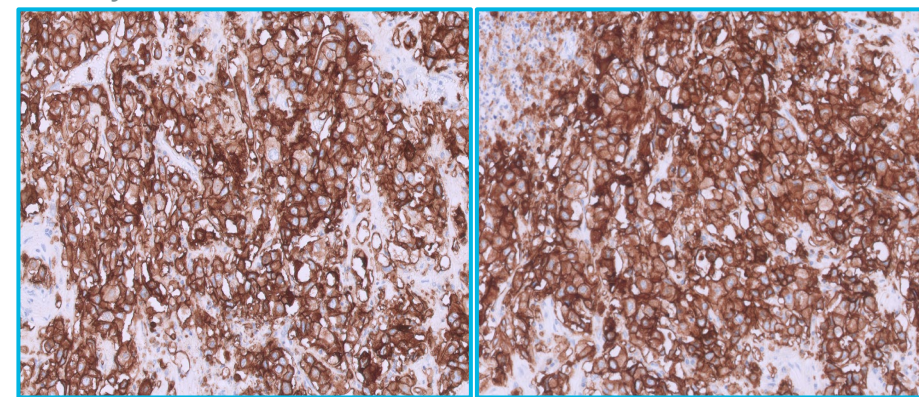


Figure 2. Front (left) and back (right) sections were assessed at a depth of 200 μ m within an FFPE block (20x).

Figure 2 is an example of a front and back pair showing IB6 High diagnostic agreement.

Intra-Case

Intra-Case testing resulted in 95.5% OA (n=44).

Table 2. Intra-Case results summary.

Specimen Distribution Summary			
Definition	Test Range	# Blocks	% Blocks
Negative	<50	2/44	4.5%
Positive	≥ 50	42/44	95.5%
Total Near Cutoff	40-60	3/44	6.8%
All Pair-Wise Comparisons Summary			
Concordant Negatives	Concordant Positives	Discordances	Total
1	41	2	44
Agreement Summary			
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)	
		Lower-bound: 2.5%	Upper-bound: 97.5%
OA	95.5%	88.6%	100.0%

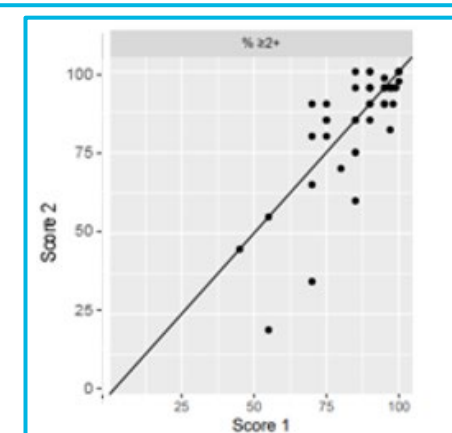


Figure 3. Intra-Case results plot. Score 1 on the x-axis represents one intra-case block IB6 score and Score 2 on the y-axis represents the second intra-case block IB6 score.

Primary vs. Metastatic

Primary vs. Metastatic testing resulted in 83.3% OA (n=30).

Table 3. Primary vs. Metastatic results summary.

Specimen Distribution Summary			
Definition	Test Range	# Blocks	% Blocks
Negative	<50	2/30	6.7%
Positive	≥ 50	28/30	93.3%
Total Near Cutoff	40-60	3/30	10.0%
All Pair-Wise Comparisons Summary			
Concordant Negatives	Concordant Positives	Discordances	Total
1	24	5	30
Agreement Summary			
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)	
		Lower-bound: 2.5%	Upper-bound: 97.5%
OA	83.3%	70.0%	96.7%

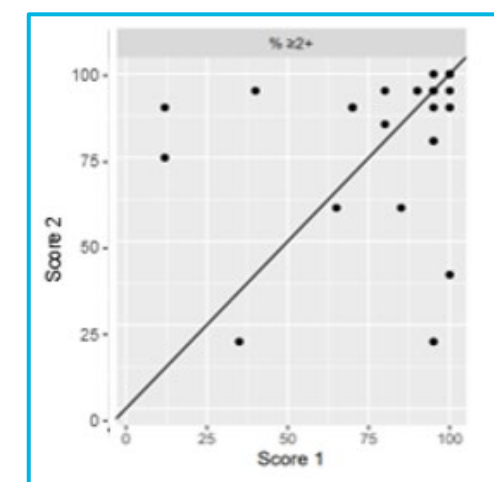


Figure 4. Primary vs. Metastatic results plot. Score 1 on the x-axis represents the primary block IB6 score and Score 2 on the y-axis represents the metastatic block IB6 score.

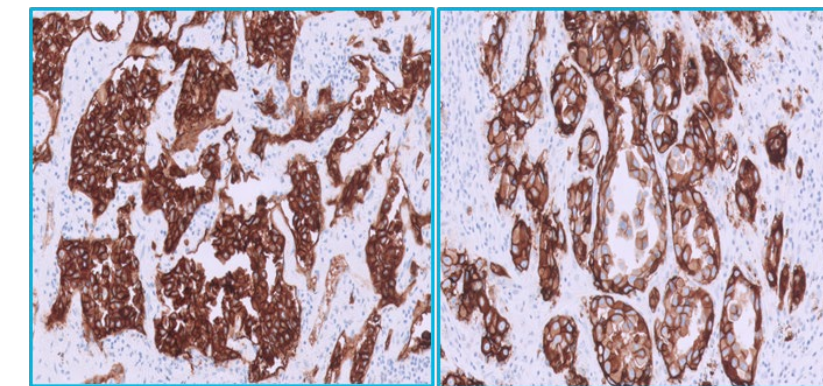


Figure 5. Lung primary tumor (left) and lymph node metastatic tumor (right) matched pair (20x). This is an example of matched pair tumors showing IB6 High diagnostic agreement.

Other Clinically Relevant Parameters

Tissue Thickness

Tissue Thickness resulted in 99.1% OA.

Table 4. Tissue Thickness results summary.

Specimen Distribution Summary				
Definition	% $\geq 2+$ Range	# Blocks	% Blocks	
Negative	<50	16/36	44.4%	
Positive	≥ 50	20/36	55.6%	
Total Near Cutoff	40-60	6/36	16.7%	
Comparisons to Consensus Summary				
Negative Consensus		Positive Consensus		Total
Concordant Negatives	Discordant Positives	Concordant Positives	Discordant Negatives	
47	1	60	0	108
Agreement Summary				
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)		
		Lower-bound: 2.5%	Upper-bound: 97.5%	
NPA	97.9%	93.8%	100.0%	
PPA	100.0%	94.0%*	100.0%*	
OA	99.1%	97.2%	100.0%	

*The percentile bootstrap method cannot compute confidence bounds if 100% agreement is observed. The Wilson Score limits are used to calculate confidence intervals for agreement parameters with point estimates equal to 100%.

Glass vs. Digital WSI

Evaluation of IB6 on glass slides compared to WSIs resulted in 98.9% OA.

Table 5. Glass vs. Digital results summary.

Specimen Distribution Summary				
Definition	% $\geq 2+$ Range	# Blocks	% Blocks	
Negative	< 50	28/60	46.7%	
Positive	≥ 50	32/60	53.3%	
Total Near Cutoff	40-60	16/60	26.7%	
Comparisons to Standard (Standard: Glass)				
Negative Standard		Positive Standard		Total
True Negatives	False Positives	True Positives	False Negatives	
81	1	97	1	180
Agreement Summary				
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)		
		Lower-bound: 2.5%	Upper-bound: 97.5%	
NPA	98.8%	96.3%	100.0%	
PPA	99.0%	96.8%	100.0%	
OA	98.9%	97.2%	100.0%	

Pre-Analytical Variables

There was no significant difference in the detection of IB6 positivity across the cold ischemia times of 0.5-72 hours and 10% neutral buffered formalin (NBF) fixation times of 6-72 hours (data not shown).

Conclusions

Integrin Beta-6 IHC 6.2A1 pharmDx (Dako Omnis) demonstrates high reproducibility within a tumor (Intra-Block and Intra-Case).

This assay showed moderate reproducibility between tumors at different sites (primary vs. metastatic), which suggests biomarker expression may vary as disease progresses. However, additional studies are needed to explore this relationship.

These findings indicate that assessment of IB6 expression can reliably be performed within tumor sites and across other clinically relevant parameters with Integrin Beta-6 IHC 6.2A1 pharmDx (Dako Omnis).

References

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Integrin Beta-6 IHC 6.2A1 pharmDx (Dako Omnis) is intended for Investigational Use Only (IUO).