



# Automated Deep Learning Quantification of Nectin-4 in Muscle-Invasive Bladder Cancer: Prognostic and Morphologic Correlates

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## BACKGROUND

- Manual IHC scoring** of antibody-drug conjugate (ADC) biomarkers (Nectin-4, TROP2, HER2, MOC31) is rarely performed in clinical settings, subject to interobserver variability
- Automated AI quantification** from HE alone to ADC marker expression levels enables scalable and reproducible companion diagnostics for ADC therapy selection
- End-to-end AI pipeline** developed for automated biomarker quantification in bladder cancer, evaluated for prognostic utility

## METHODS

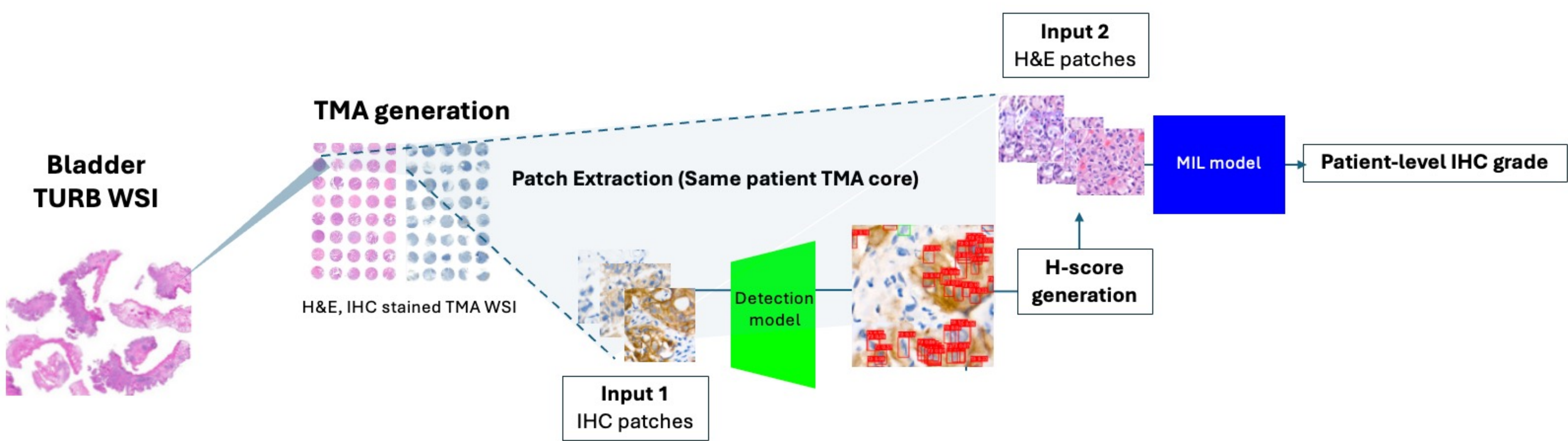


**Figure 1. Schematic overview of the AI pipeline.** IHC-stained whole slide images from TMA are processed through YOLOv8 object detection to generate automated H-scores. In parallel, H&E-stained patches from the same patients serve as input to an attention-based MIL model for H&E-based biomarker prediction. IHC, immunohistochemistry; TMA, tissue microarray; MIL, multiple instance learning; GradCAM, gradient-weighted class activation mapping.

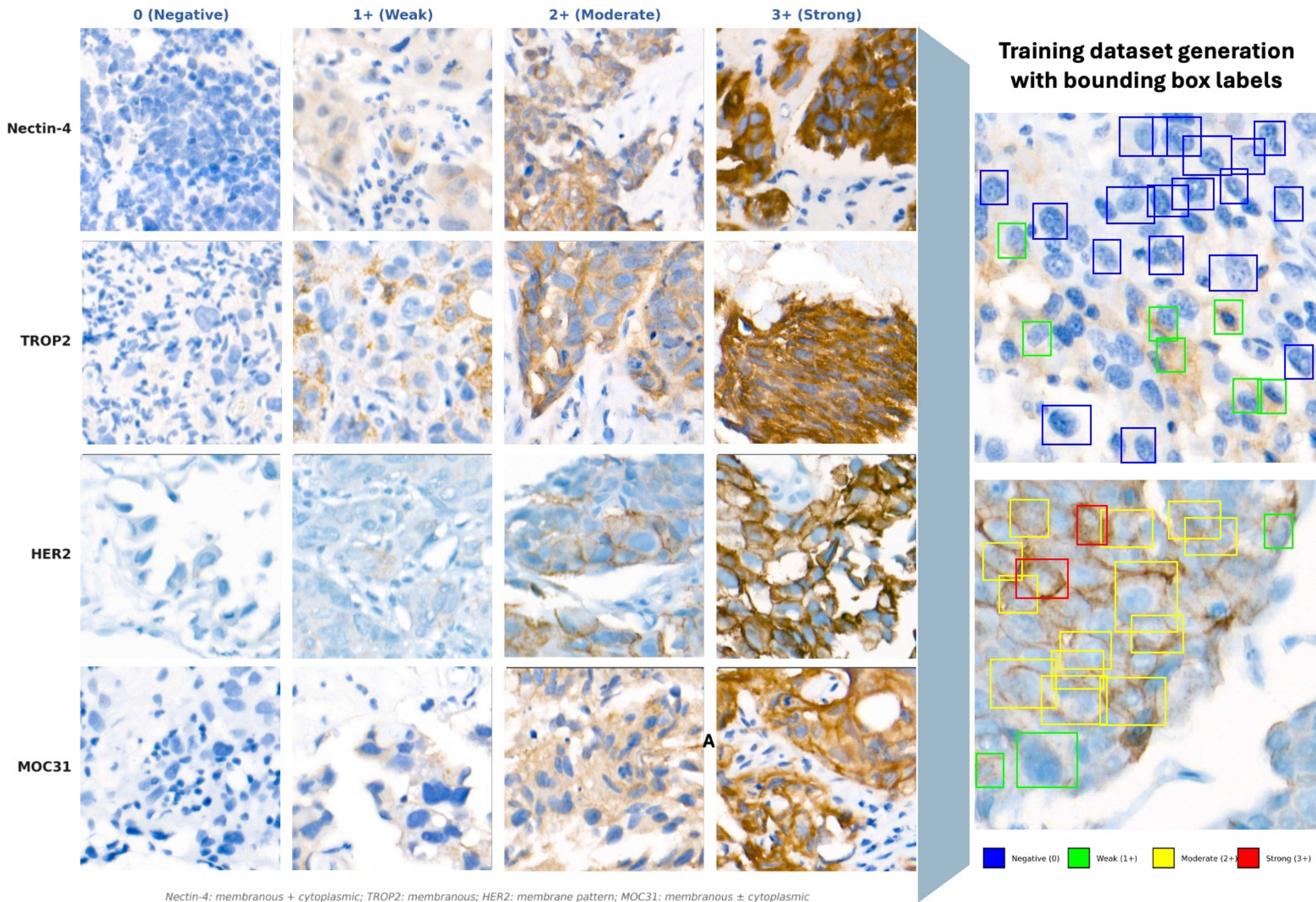


**Figure 2. Representative immunohistochemical staining patterns by intensity grade**

Left panels show tumor patches selected to illustrate staining intensity level (0, 1+, 2+, 3+) based on the predominant cell population. Nectin-4: membranous and cytoplasmic staining; TROP2: membranous staining; HER2: membranous staining; MOC31: membranous with or without cytoplasmic staining. Right panels demonstrate bounding box annotations for YOLOv8 training, with cells color-coded by staining intensity (blue: 0, negative, green: 1+, weak, yellow: 2+, moderate, red: 3+, strong).

- H&E patches extracted from same patient's TMA core as IHC sections → input to an attention-based MIL model
- ResNet-18 encoder (pretrained on ImageNet) extracts 512-dimensional feature vector per patch
- Attention mechanism assigns learnable importance weights to individual patches, focusing on morphologically informative regions
- Aggregated patient-level representation passed through fully connected layers → final IHC grade classification
- Agreement between AI-derived and pathologist-assigned IHC grades was evaluated using Cohen's weighted kappa ( $\kappa$ ).
- GradCAM applied to visualize patch-level contributions; high-attention regions reviewed by pathologist for morphological correlation

## RESULTS

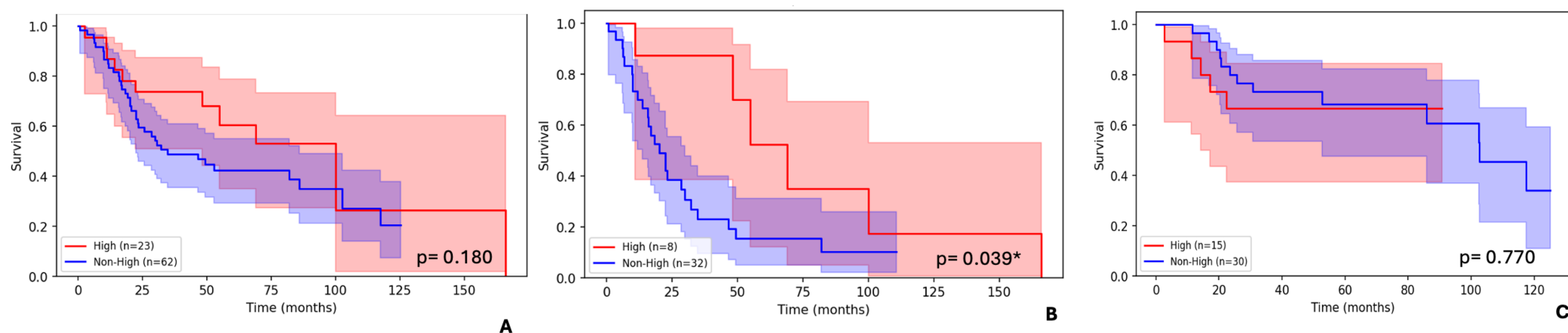
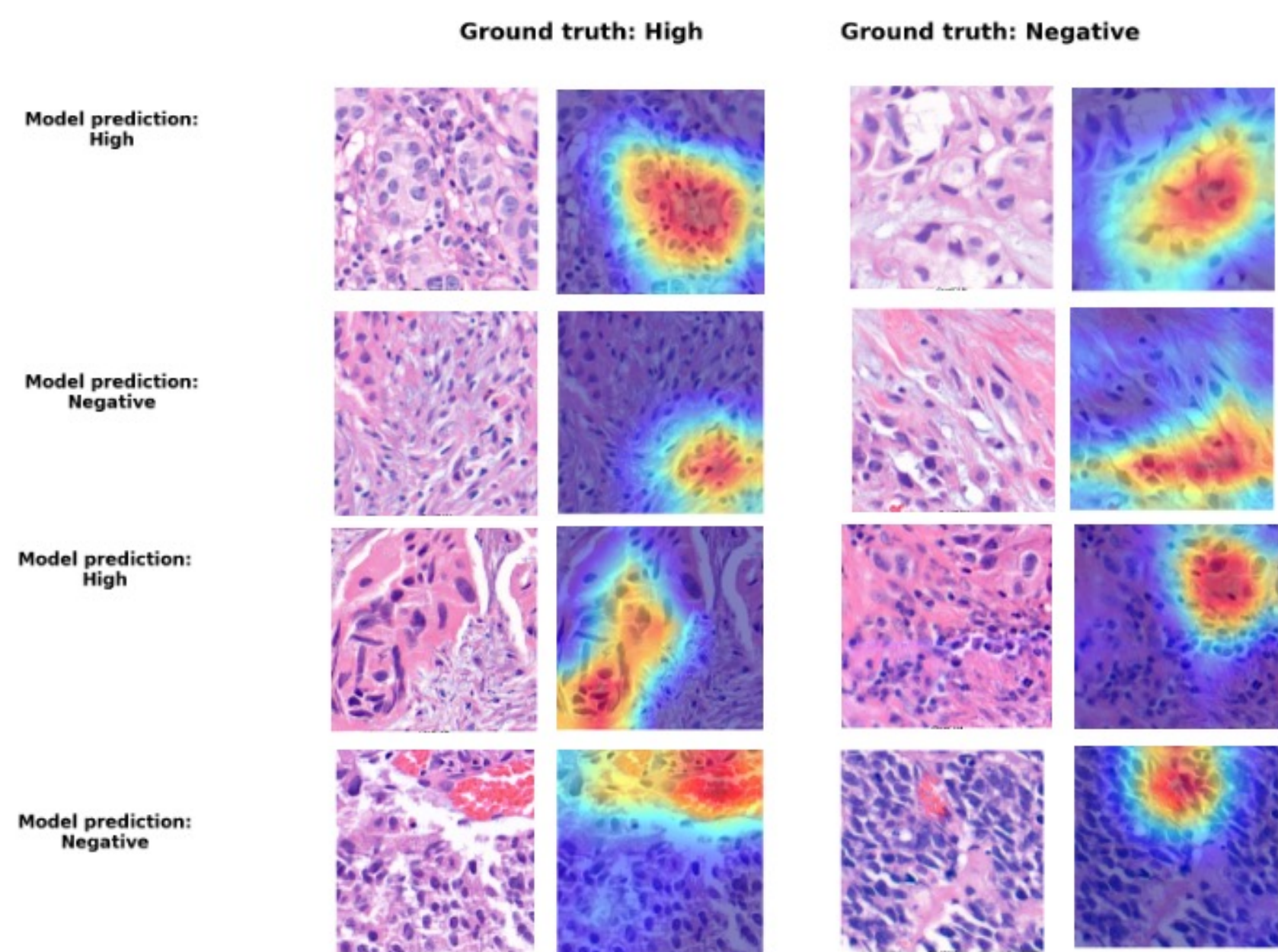


**Figure 3. Kaplan-Meier curves for overall survival stratified by Nectin-4 IHC expression assessed by AI model (High vs. Non-High).** (A) Overall cohort (n=85). (B) Muscle-invasive bladder cancer subgroup (n=40); Nectin-4 high expression is associated with significantly improved overall survival (p=0.039). (C) Non-muscle-invasive subgroup (n=45). Log-rank test.

**Table. MIL Model Performance : H&E-based Biomarker Prediction**

| Biomarker | Accuracy | Sensitivity | Specificity | PPV*         |
|-----------|----------|-------------|-------------|--------------|
| Nectin-4  | 64.7%    | 34.8%       | 75.8%       | <b>87.0%</b> |
| TROP2     | 46.4%    | 57.4%       | 32.4%       | <b>84.6%</b> |
| HER2      | 59.5%    | 22.2%       | 87.5%       | <b>64.3%</b> |
| MOC31     | 54.1%    | 35.7%       | 63.2%       | <b>32.3%</b> |



PPV\* = positive predictive value for therapy-eligible expression: Nectin-4/TROP2 High; HER2 Positive/Equivocal. PPV\* 87% for Nectin-4 demonstrates H&E-based screening feasibility.

**Figure 4. GradCAM attention heatmaps for MIL-based biomarker prediction** across four markers (Nectin-4, TROP2(not shown), MOC31(not shown) HER2), stratified by ground truth expression (High vs. Negative) and model prediction outcome. Nectin-4<sup>Hifh</sup> is associated with well-differentiated papillary architecture; HER2<sup>+</sup> with high nuclear-to-cytoplasmic ratio in invasive tumor clusters.

## DISCUSSION & CONCLUSION

- Pathologist-level IHC quantification:** YOLOv8 achieves near-perfect agreement ( $\kappa \geq 0.917$ ) for all four ADC biomarkers, enabling reproducible automated scoring
- AI-derived Nectin-4 high expression** independently predicts better OS in MIBC (HR 0.37, 95% CI 0.14–0.98, p=0.046)
- H&E-based screening feasibility:** MIL model achieves 87% PPV\* for therapy-eligible Nectin-4 expression, offering a scalable pre-screening approach without IHC
- Morphologic correlates:** GradCAM reveals papillary architecture as a key feature for Nectin-4 positivity; high N:C ratio for HER2 positivity, regardless of ground truth label
- Clinical implication:** This pipeline provides a scalable, reproducible approach for ADC therapy candidate identification, particularly applicable to small TURB biopsy specimens

## CONTACTS

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