PRODUCT SUPPORT

Introduction to EV Antibody Biomarkers

Utility of Biomarkers
- EV1 alone maps the tumour margin, highlighting the migrating fronts
- EV1 and EV3 are used in combination to identify benign regions of the prostate
- EV2 and EV3 are used in combination to interpret complex morphology, thereby distinguishing low-grade and high-grade cancer
- EV3 can highlight inconspicuous lesions of cancer

### Using the biomarkers to interpret complex morphology
- When EV2 is punctate and supranuclear, but EV3 labelling is below baseline threshold (basal cell staining intensity), this confirms low grade cancer (well-formed glands)
- When EV2 labelling is granular and no longer supranuclear, but EV3 labelling is equal to or above the baseline threshold (basal cell staining intensity), this confirms high grade cancer (poorly formed glands)
- In areas of cancer where EV2 and EV3 interpretation is unequivocal, refer only to EV3
- EV3 can highlight small pockets (including single cells) of cancer

Biomarker Labelling Distribution
- EV1
  - EV1 labels basal cells (baseline threshold)
  - EV1 can label nuclei in PIN glands
  - EV1 labels luminal cells in cancer; diluse and cytoplasmic
  - EV1 labels endothelial cells
  - EV1 labels inflammatory cells
  - EV1 labels ganglion/nerve cell bodies
  - EV1 labels seminal vesicle structures

- EV2
  - EV2 has minimal labelling in benign luminal cells; punctate and supranuclear
  - EV2 does not label basal cells
  - EV2 labels luminal cells (punctate and supranuclear) in PIN tissue
  - In comparison to benign glands, EV2 labelling is abundant in luminal cells (punctate and supranuclear) in low grade cancer
  - In comparison to low grade cancer, EV2 labelling is granular and no longer supranuclear in its position in high grade cancer

- EV3
    - EV3 labels basal cells (baseline threshold, (basal cell staining intensity)), while luminal cells display no labelling
    - EV3 labels luminal cells in cancer; granular and cytoplasmic
    - In comparison to low-grade cancer, EV3 labelling is stronger in high-grade cancer
    - EV3 labels basal cell hyperplasia
    - EV3 labels ganglion/nerve cell bodies
    - EV3 labels single cancer cells (cytoplasmic) and plasma cells (membranous)

Example of the Utility of Biomarker Labelling In Interpreting Complex Prostate Cancer Morphology
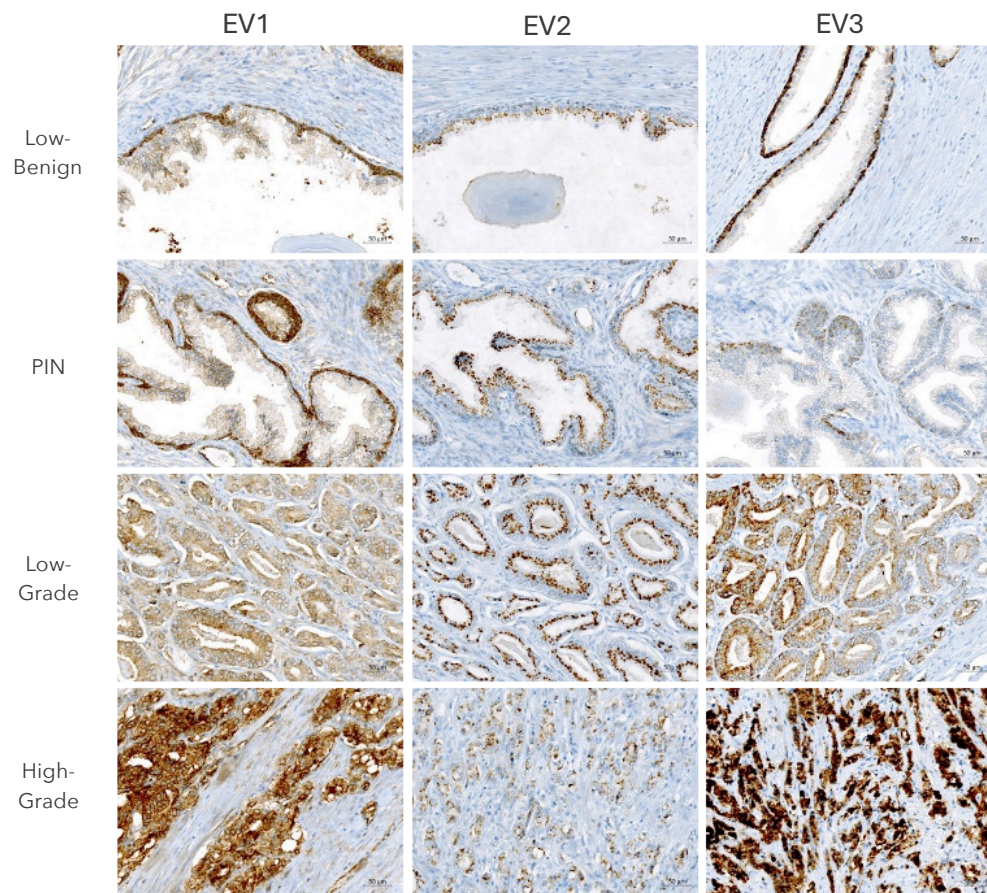
Utility of the Three Biomarkers APPL-1, Sortilin-1 and Syndecan-1 for Cancer Detection and Grading

- The combination of APPL-1 and Syndecan-1 can be used to define the onset of cancer (PIN tissue formation). APPL-1 undergoes a distribution change from clearly defined basal cell staining to a cytoplasmic distribution in PIN tissue, often with the staining of nuclear inclusions in selected cells. Syndecan-1 has a very intense basal cell staining pattern in benign tissue but is lost as PIN tissue forms. This combination of APPL-1 and Syndecan-1 electively identifies prostate cancer initiation.

- APPL-1 increases in expression with the formation of establishment cancer and Sortilin-1 exhibits a specific polarised distribution. This combination of biomarkers identifies lower grade cancers and at this stage the cancer may have developed some Syndecan-1 staining. This combination of the three biomarkers electively identifies an early stage of cancer progression.

- APPL-1 is optimal for scanning large areas of tissue to identify the cancer and its boundaries, and the stroma is not stained (no background); but it is difficult to distinguish whether glands are cribriform/fused glands, compared to closely arranged GG3 glands. Syndecan-1 provides confirmation for this staining and is frequently distributed in the same manner as APPL-1 even though it is more distinct and granular. However, Sortilin-1 clearly distinguished between cribriform and fused glands.

    - Sortilin-1 is optimal for this visualisation because the staining is polar, and the cells and borders are better distinguished, demonstrating clearly separated GG3 glands compared to fused/cribriform glands.

- In Grade 4/5 cancers where cells proliferate to form fused glands/sheets, APPL1 cannot differentiate the fused glands versus sheets of cells, whereas Syndecan-1 indicates the presence of fused glands and combined with Sortilin-1 confirms this morphology.

    - Because APPL1 stains the stroma and appears more sheet like in all of the cores, whereas Sortilin-1 and Syndecan-1 do not, the spaces (indicating fused glands) or stroma (indicating sheets) are clearly distinguished with this combination of biomarkers.

- While there is considerable co-staining of the three biomarkers and higher amounts of APPL-1 and Syndecan-1 signify advanced cancer; the specific pattern of intense APPL-1 staining and intense Syndecan-1 staining, but with little or no Sortilin-1 staining is observed for patients at high risk of clinical recurrence (metastasis).

- The combination of APPL-1 (due to its increasing intensity as the cancer progresses to an advanced stage) and Syndecan-1 (due to its high intensity staining in advanced cancer and in migrating cancer cells) enables the elective detection of advanced cancer; this is balanced against Sortilin-1 expression to make decisions on how far the cancer has progressed. This combination of biomarker enables:

  - More accurate detection of cancer facilitating optimal identification of the cancer in patients previously graded Gleason ≤ 6 or Epstein group 1
  - Detection of the presence of cancer at a distance from the primary pathogenesis
  - More definitive identification of advanced cancer in Gleason grade 7 or Epstein group 1 and 2 patients
  - Specific recognition of patients at risk of clinical recurrence

Interpretation with Conventional Microscopy

1) **First, evaluate the APPL1 stained section at low power (4x) magnification. This may highlight areas** of cancer and its boundaries. Areas of cancer are obvious with APPL1 at low power and staining intensity may increases as the cancer progresses to an advanced stage.

2) **Next, utilise both sortilin-1 and syndecan-1 together to score areas of low-grade (GG3) and high-grade (GG4) cancer, respectively.**

   a. **Low power magnification with Syndecan-1 easily detects benign glands with strong basal cell staining and absent or weak staining in the secretory epithelial cell layer.** Syndecan-1 staining presents with punctate and moderate staining intensity in areas of GG3 and GG5 cancer. Syndecan-1 generally increases in intensity in GG4. Areas of advanced cancer with migrating fronts may show strong characteristic staining of Syndecan-1, while also highlighting single cancer cells. Strong Syndecan-1 staining may represent areas high-grade cancer.

   b. **SORT1 staining presents with strong staining in low-grade cancer (GG3) and sometimes may present with reduced staining in areas of high-grade cancer (>GG3).** In areas of low-grade cancer, Sortilin-1 staining is very polar and is distributed in a supranuclear position. Loss of Sortilin-1 staining intensity and loss of polarity of staining may sometimes indicate progression to more advanced cancer (>GG3).

Staining Patterns Not Associated with Tumour Cells

1) EV1 intensely stain basal cells thus highlighting benign glands by staining basal cells and sometimes secretory cells to a less-extent. EV1 also stains a subset of WBC infiltrate.

2) EV2 demonstrates supranuclear staining in PIN tissue, where progressive increase in staining is observed with loss of basal cells and development of PIN tissue.

3) EV3 intensely stains basal cells in benign glands and basal cell hyperplasia. The staining is punctate cytoplasmic and highlights benign glands. PIN tissue staining upregulated. Infiltrate can sometimes stain WBC infiltrate.

Current GG system

- Grade Group 1 (Gleason score ≤6) – Only individual discrete well-formed glands

- Grade Group 2 (Gleason score 3+4=7) – Predominantly well-formed glands with a lesser component of poorly-formed/fused/cribriform glands (evident loss of luminal area)

- Grade Group 3 (Gleason score 4+3=7) – Predominantly poorly-formed/fused/cribriform glands with a lesser component of well-formed glands†

- Grade Group 4 (Gleason score 8) - Only poorly-formed/fused/cribriform glands or - Predominantly well-formed glands with a lesser component lacking glands†† or - Predominantly lacking glands with a lesser component of well-formed glands††

- Grade Group 5 (Gleason scores 9-10) – Lacks gland formation (or with necrosis) with or w/o poorly-formed/fused/cribriform glands†

Key Prostate Cancer Pathologies Outlined by APPL1, Sortilin-1 And Syndecan-1 Biomarkers for Utility in ISUP Grade Grouping

Row 1: Benign prostatic gland stained with routine H&E, dual labelled with p63 + 34βE12 and AMACR, as well as immunolabelled with APPL1, Sortilin-1 and Syndecan-1.

Row 2: Low grade prostatic intraepithelial neoplasia stained with routine H&E, dual labelled with p63 + 34βE12 and AMACR, as well as immunolabelled with APPL1, Sortilin-1 and Syndecan-1.

Row 3: High grade prostatic intraepithelial neoplasia stained with routine H&E, dual labelled with p63 + 34βE12 and AMACR, as well as immunolabelled with APPL1, Sortilin-1 and Syndecan-1.

Row 4: Well-formed cancer glands stained with routine H&E, dual labelled with p63 + 34βE12 and AMACR, as well as immunolabelled with APPL1, Sortilin-1 and Syndecan-1. Row 5: Poorly-formed cancer glands stained with routine H&E, dual labelled with p63 + 34βE12 and AMACR, as well as immunolabelled with APPL1, Sortilin-1 and Syndecan-1.

Row 6: Cribriform glands stained with routine H&E, dual labelled with p63 + 34βE12 and AMACR, as well as immunolabelled with APPL1, Sortilin-1 and Syndecan-1.
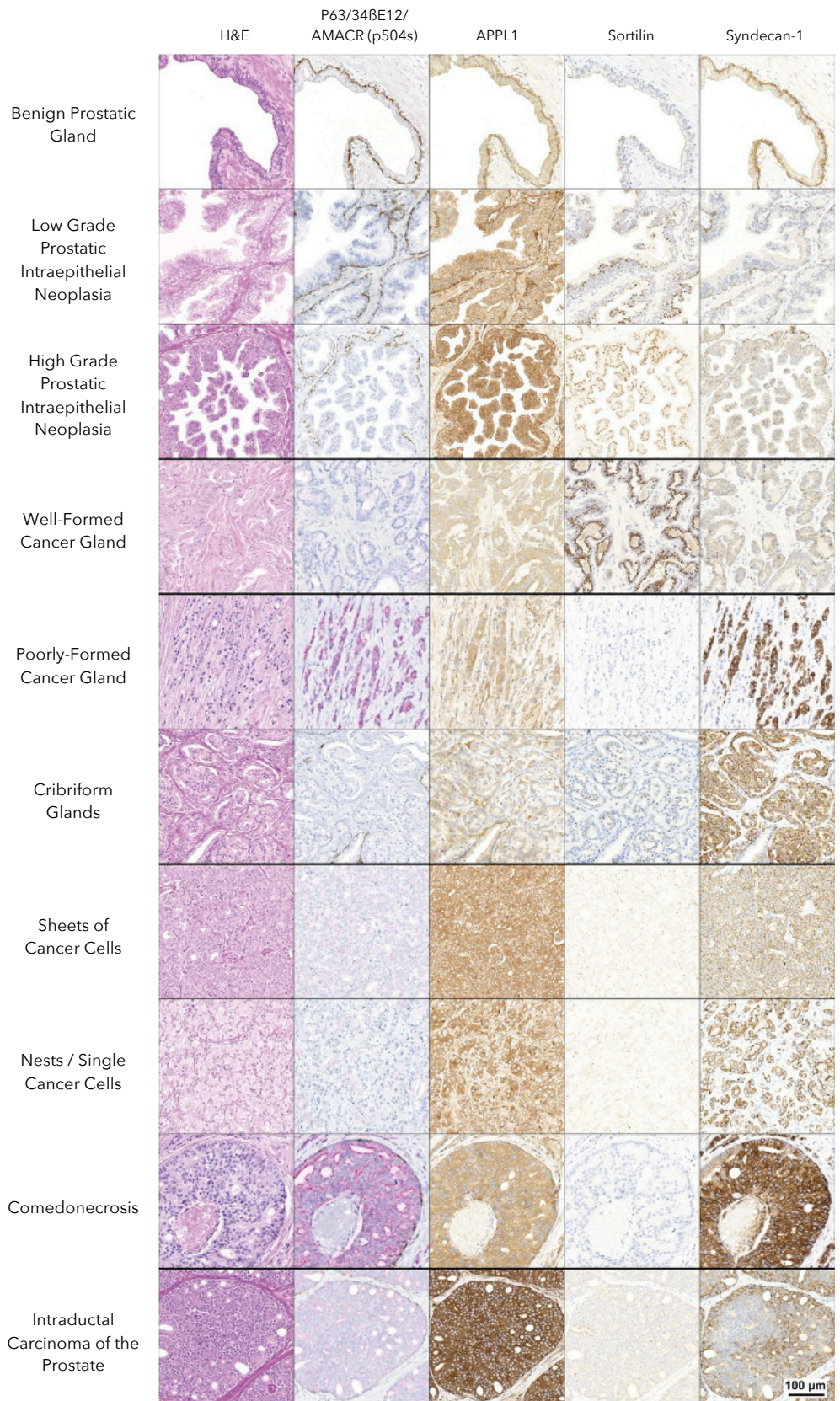
Row 7: Sheets of cancer cells stained with routine H&E, dual labelled with p63 + 34βE12 and AMACR, as well as immunolabelled with APPL1, Sortilin-1 and Syndecan-1.

Row 8: Nests and single cancer cells stained with routine H&E, dual labelled with p63 + 34βE12 and AMACR, as well as immunolabelled with APPL1, Sortilin-1 and Syndecan-1.

Row 9: Intraductal carcinoma of the prostate stained with routine H&E, dual labelled with p63 + 34βE12 and AMACR, as well as immunolabelled with APPL1, Sortilin-1 and Syndecan-1.

Row 10: Comedo-necrosis stained with routine H&E, dual labelled with p63 + 34βE12 and AMACR, as well as immunolabelled with APPL1, Sortilin-1 and Syndecan-1.

APPL1 displays brown insoluble precipitate throughout the cytoplasm of the basal cells in benign prostatic glands before it transfers into the cytoplasm of dysplastic secretory cells. The dysplastic cells increase in intensity as neoplasia develops with the highest intensity in advanced cancer. Sortilin-1 punctate labelling is observed within the neoplastic cells in PIN tissue and in early-stage cancer. The punctate labelling is polarized on the apical side adjacent to the nuclei. As the cancer advances the intensity and polarity of the labelling reduces before it is completely absent in high grade cancer. In contrast, Syndecan-1 illustrates fine cytoplasmic labeling throughout all neoplastic cells in all key pathologies. As the cancer advances, the labelling intensity increases while intraductal carcinoma foci label patchy with Syndecan-1. Similar to APPL1, Syndecan-1 is also present within basal cells of benign glands.

	H&E	P63/34ßE12/ AMACR (p504s)	APPL1	Sortilin	Syndecan-1
Benign Prostatic Gland					
Low Grade Prostatic Intraepithelial Neoplasia					
High Grade Prostatic Intraepithelial Neoplasia					
Well-Formed Cancer Gland					
Poorly-Formed Cancer Gland					
Cribriform Glands					
Sheets of Cancer Cells					
Nests / Single Cancer Cells					
Comedonecrosis					
Intraductal Carcinoma of the Prostate					

100 µm

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Stratification Of ISUP Grade Group with APPL1, Sortilin and Syndecan-1 In Prostate Core Biopsies
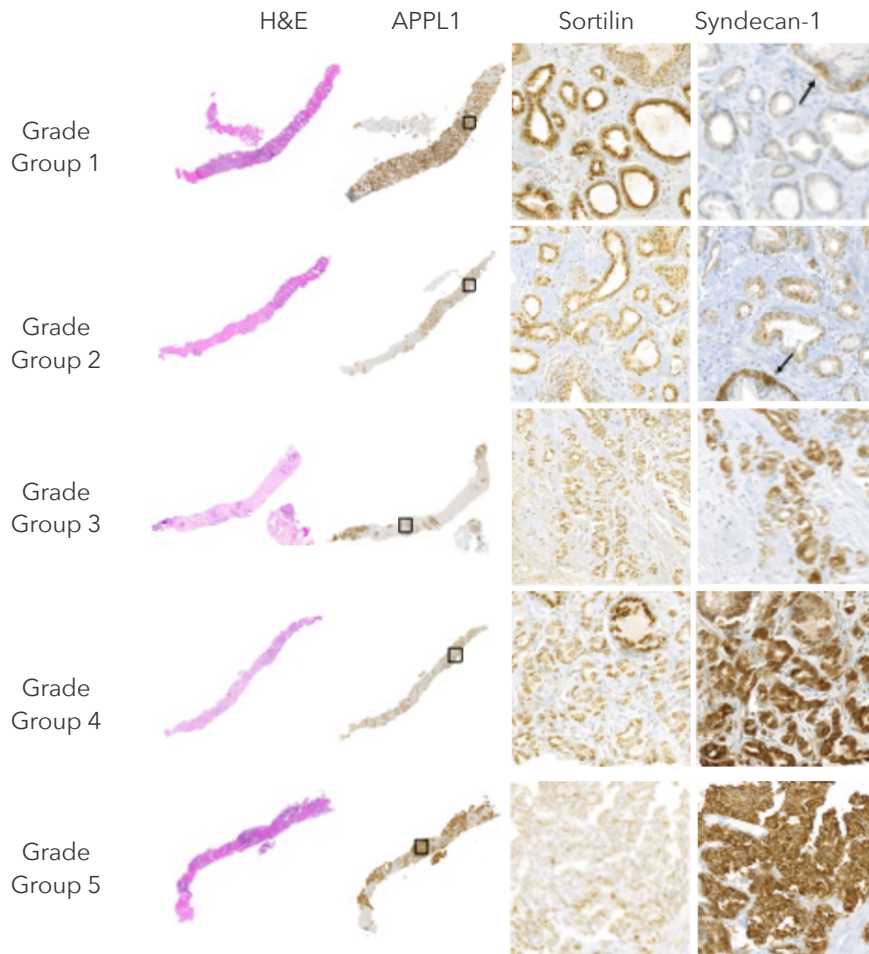
Left: Haematoxylin and eosin stain and APPL1 labelling at low power.

Right: High power images of square regions of Sortilin and Syndecan-1.

In comparison to H&E, APPL1, at low power, can highlight regions of interest to be assessed at high power with Sortilin and Syndecan-1 for stratification of ISUP grade groups. Grade Group 1; Sortilin displays intense polar labelling in a supranuclear position while Syndecan-1 labelling is absent.

Grade Group 2; Sortilin labelling is still polar and in a supranuclear position, and Syndecan-1 displays low labelling intensity in cytoplasm of cancer cells (below basal cells threshold). Grade Group 3; Sortilin labelling displays loss in polarity, no longer supranuclear, while Syndecan-1 cytoplasmic intensity increases (at or above basal cells threshold). Grade Group 4; Sortilin displays both loss in intensity and polarity, while Syndecan-1 labelling is equal or above threshold (basal cells labelling). Grade Group 5; Syndecan-1 displays intense labelling in cytoplasm of cancer cells (equal to or above basal cells threshold) while Sortilin labelling is absent.