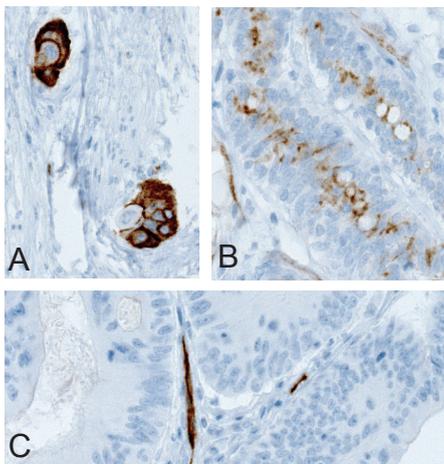


## PODXL - an independent biomarker of poor prognosis in cancer

### Cancer

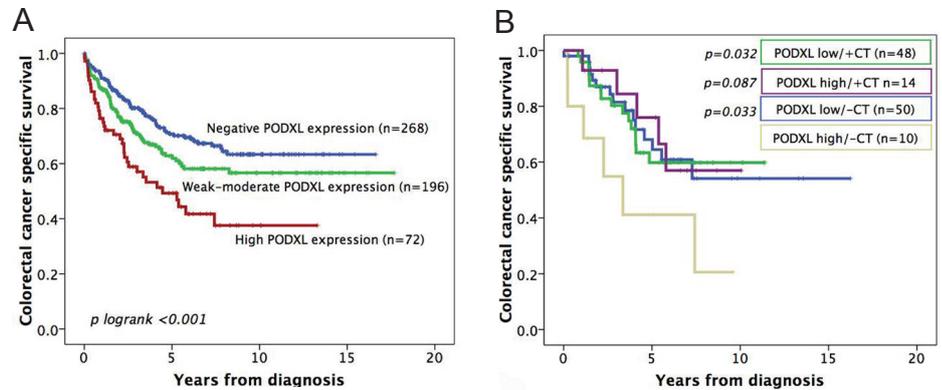
Cancer is a major health problem throughout the world. In 2008, there were an estimated 12.7 million new cancer cases world-wide, and 7.6 million deaths that could be attributed to cancer. Due to a world population with an increased life-expectancy, there is no reason to expect a decline in cancer incidence in the near future.

Cancer, though often denoted as a singular disease, is truly a multitude of diseases. This understanding has evolved over the years, but still many patients are not receiving optimal treatment for their disease. For cancer patients to receive a more individualized treatment, there is a need for new and better ways to stratify patients. The classical, pathology based prognostic factors such as stage and grade of the tumor are insufficient for a correct estimation of patient prognosis. Additional information from immunopathology biomarkers promise to substantially improve this estimation, ultimately leading to a more individualized treatment, thus avoiding both under- and over treatment of patients.



**Figure 1**

A) Membranous, B) non-membranous/cytoplasmic and C) absence of immunoreactivity in colorectal tumor samples following IHC staining with Anti-PODXL (AMAb90667) antibody.



**Figure 2**

A) Kaplan-Meier analysis of colorectal cancer-specific survival of patients with tumors expressing no, weak/moderate, or high levels of PODXL protein. B) Kaplan-Meier analysis of colorectal cancer-specific survival, where patients were divided into groups according to whether they received adjuvant chemotherapy (CT) or not, as well as the level of tumor PODXL expression (high or low).

### PODXL

Podocalyxin (PODXL) is a transmembrane protein that is involved in cell-cell interaction. In normal tissue, the protein is expressed in the kidney glomerular podocytes, where it plays an important part in maintaining filtration pathways, and in endothelial cells in blood vessels. PODXL is overexpressed in several types of cancer, e.g. breast, prostate, and testicular cancer. The PODXL protein was identified on the Human Protein Atlas (proteomics.org)<sup>1,2,3,4</sup> as a potentially interesting testicular cancer biomarker, and has later been found to be a prognostic biomarker in both colorectal<sup>5</sup> and urothelial cancer<sup>6</sup>.

### PODXL in colorectal cancer

Colorectal cancer is one of the most common types of cancer. Each year, approximately one million new cases are detected, and approximately 600,000 deaths can be contributed to this disease worldwide. Today, surgery is the only curative treatment for colorectal cancer, but adjuvant treatment may significantly improve patient survival. For adjuvant treatment to be successful, however, it is important to correctly identify patients that will benefit from treatment. Adjuvant treatment is currently recommended for patients with stage III and high-risk stage II disease. For patients with stage II colorectal cancer, it is thus of utmost importance to find biomarkers that can separate high-risk disease from low-risk disease.

PODXL protein expression has been analyzed by immunohistochemistry (IHC) in three different colorectal cancer patient cohorts<sup>5</sup>. High membranous PODXL expression in the tumor was shown to be an independent predictor of poor prognosis in all three patient cohorts. In Figure 1, IHC stainings of membranous versus no or non-membranous positivity using a monoclonal Anti-PODXL antibody are presented. There was no association between PODXL expression and age at diagnosis, gender, or tumor location in any of the cohorts studied.

Figure 2A shows survival of colorectal cancer patients according to membranous PODXL expression in the tumors. A high membranous PODXL expression correlated with a reduced survival in all cohorts. Patients with tumors expressing high membranous levels of PODXL that were treated with adjuvant chemotherapy (CT) had similar colorectal cancer specific survival (CCSS) to patients with PODXL-low tumors. Untreated patients with PODXL-high tumors had shorter CCSS than all the other patient groups (Figure 2B).

These results suggest that patients with tumors expressing high membranous levels of PODXL would benefit from adjuvant chemotherapy.

### PODXL in urothelial cancer

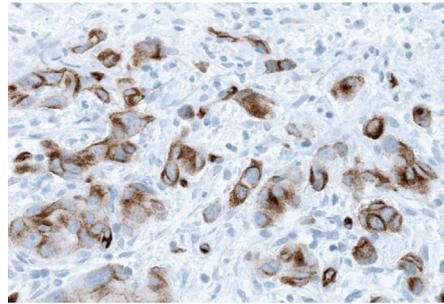
A major clinical problem in urothelial cancer is the identification of high-risk patients among those diagnosed with T1 disease (where the tumor has invaded connective tissue but not yet muscle). For these stage T1-patients, the prognosis is generally good, but nearly one third will still eventually require cystectomy after failure of other therapies. To identify these patients at an early stage would mean a great improvement for patients as well as society. Due to high recurrence rates, and need for invasive monitoring of the disease, the health care cost per patient is high for urothelial cancer. For patients with muscle-invasive disease, prognostic markers that could guide in the choice of treatment and/or treatment intensity would also mean an improvement in patient care.

The association between membranous PODXL expression and reduced survival was confirmed in both Cox univariable analysis and multivariable analysis adjusted for age, gender, T-stage and grade. When analyzing membranous PODXL expression in the subgroup of patients with stage Ta (non-invasive) and T1 tumors, it was found that membranous PODXL expression was associated with increased risk of disease progression as well as increased risk of death from disease in this group of patients. Figure 3 shows an example of membranous PODXL expression from a urothelial carcinoma sample using immunohistochemistry.

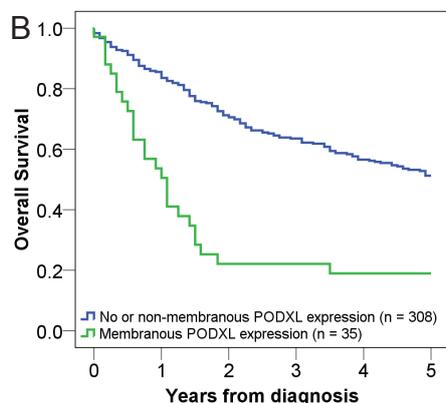
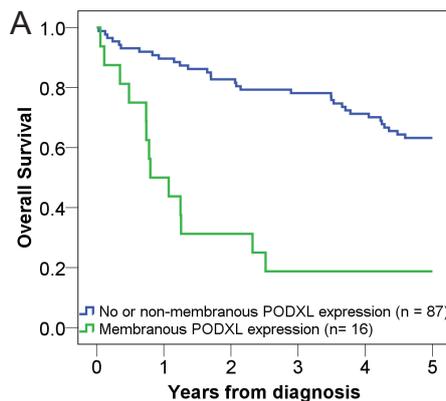
PODXL protein expression has been analyzed by IHC in two different urothelial cancer patient cohorts (Cohort I, n=110 and Cohort II, n=344 respectively). Compared to no or non-membranous expression, membranous PODXL expression in the tumor was shown to be associated with a reduced 5-year overall survival in both patient cohorts (Figure 4A and B).

### Summary

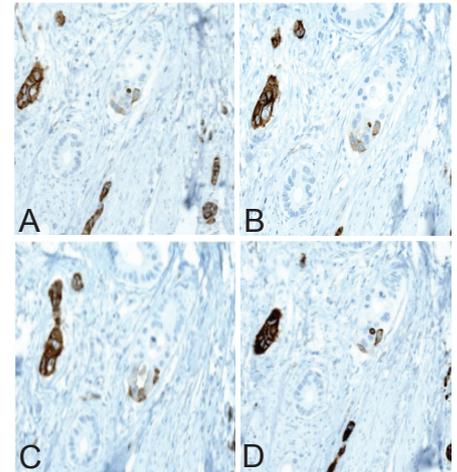
- There is a great need today for novel biomarkers capable of distinguishing between different types, stages, and forms of both colorectal and urothelial cancer.
- PODXL has been found to be a prognostic biomarker in colorectal and urothelial cancer, with high membranous PODXL expression being an independent predictor of poor prognosis.
- Analyzing PODXL expression could stratify colorectal cancer patients into those that should receive chemotherapy and those that may be spared adjuvant treatment.



**Figure 3**  
Membranous PODXL expression in urothelial cancer obtained by IHC analysis using the Anti-PODXL antibody AMAb90643.



**Figure 4**  
Kaplan-Meier estimates of 5-year Overall Survival (OS) according to PODXL expression in (A) Cohort I and (B) Cohort II.



**Figure 5**  
Immunohistochemical staining of PODXL protein in colorectal tumor tissue using A) HPA002110, B) AMAb90643, C) AMAb90644 and D) AMAb90667 antibodies.

### Monoclonal Anti-PODXL antibodies

Monoclonal mouse Anti-PODXL antibodies; AMAb90667, AMAb90643 and AMAb90644, have been developed by Atlas Antibodies, (Stockholm, Sweden, atlasantibodies.com). These monoclonal antibodies have been extensively evaluated by IHC. The achieved IHC staining pattern in urothelial and colorectal cancer tissues have been compared to the staining pattern of the corresponding polyclonal Anti-PODXL antibody (HPA002110) used in the colorectal studies described here. Figure 5 shows consecutive sections of colorectal cancer tissue using the different Anti-PODXL antibodies.

### References:

- 1) Uhlén M *et al.* Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol.* 2010 28(12):1248-50.
- 2) Berglund L. *et al.* A gene-centric human protein atlas for expression profiles based on antibodies. *Molecular & Cellular Proteomics.* 2008 7:2019-2027.
- 3) Asplund A. *et al.* Antibodies for profiling the human proteome - The Human Protein Atlas - a resource for cancer research. *Proteomics.* 2012 Jul;12(13):2067-77.
- 4) Pontén F, Jirstrom K, Uhlén M. The Human Protein Atlas - a tool for pathology. *J Pathology* 2008 216(4):387-93.
- 5) Larsson A. *et al.* Overexpression of podocalyxin-like protein is an independent factor of poor prognosis in colorectal cancer. *Br J Cancer* 2011 105(5):666-72.
- 6) Boman K. *et al.* Membranous expression of podocalyxin-like protein is an independent factor of poor prognosis in urothelial bladder cancer. *Br J Cancer* 2013 108, 2321-2328.

