Vn96* Peptide: a novel tool for convenient and efficient enrichment of urinary eMVs containing informative prostate cancer protein and mRNA biomarkers

INSTITUT ATLANTIQUE DE RECHERCHE SUR LE CANCER
ATLANTIC CANCER RESEARCH INSTITUTE

Michelle Davey[1], Michelle Caissie[1], Sarah Melville[1], Sébastien Fournier[1], Marc Savoie[2], Guy Breault[2] and Rodney J. Ouellette[1]

1 Atlantic Cancer Research Institute, Moncton, New Brunswick, Canada; 2 Dr. Georges-L.-Dumont University Hospital Centre, Urology Depart., Moncton, New Brunswick, Canada

***Patent Pending**

Introduction

Early detection of prostate cancer (PCa) currently relies on serum PSA and digital rectal examination (DRE), both of which lack diagnostic specificity and prognostic value. Hence, there is a great demand for new and better PCa biomarkers. Urinary extracellular microvesicles (eMVs) have valuable potential as a novel, non-invasive and enriched source of biomarkers. Conventional means of eMV isolation involve time-consuming ultracentrifugation (UCF) or expensive commercial methods. Our group has developed a fast, simple and cost-effective method for enrichment of eMVs from urine using a peptide (Vn96) with affinity for heat shock proteins.

Materials and Methods

Post-DRE urine samples were collected from patients scheduled for prostate biopsy. eMVs were isolated in parallel using UCF and Vn96 affinity peptide techniques. Western blotting was used to assess and compare the presence of classical eMV markers (e.g. CD9, ALIX, HSP70) and prostate-specific markers (e.g. FOLH1, PSA). Total + small RNA was extracted from UCF and Vn96 eMVs, and from exfoliated cells (in urine sediments). RT-qPCR, employing a multiplexed pre-amplification step, was used to assess the expression of known urinary PCa markers (*e.g. PSA, PCA3*) and a panel of five genes of interest to our group.

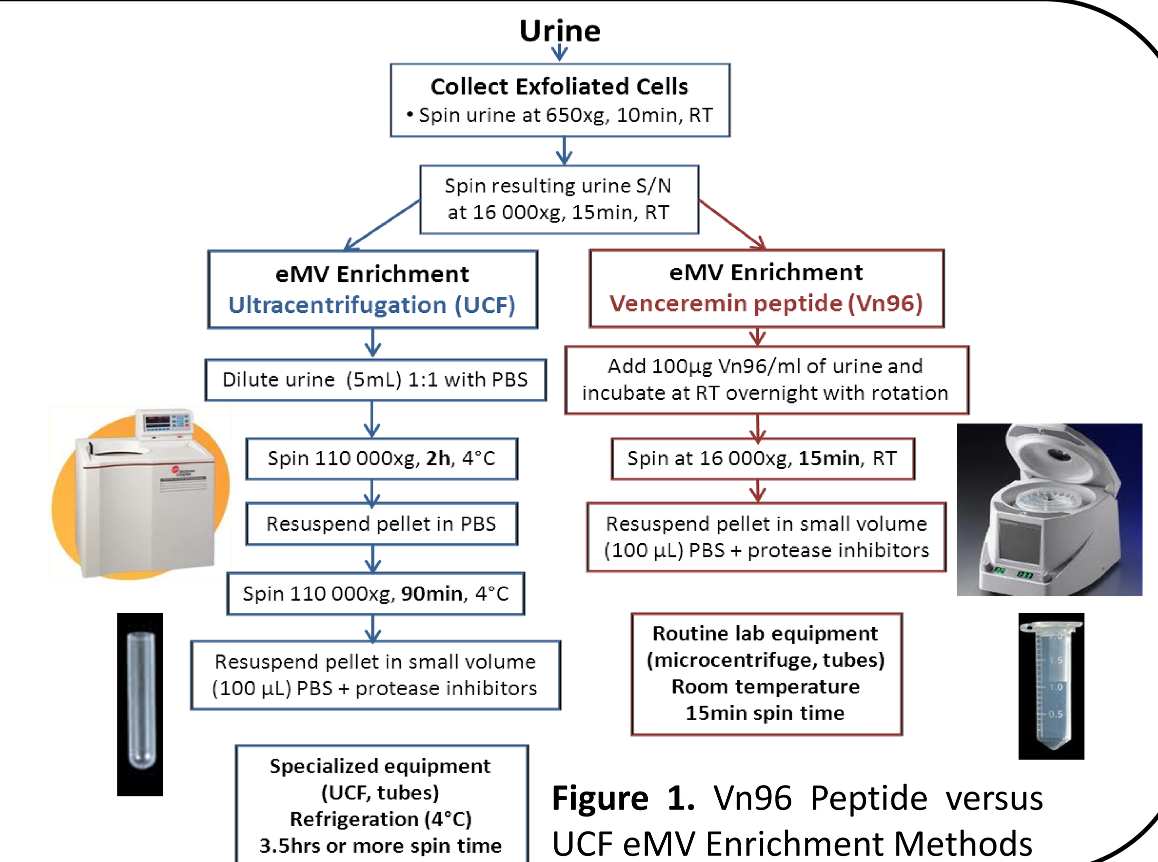

Figure 1. Vn96 Peptide versus UCF eMV Enrichment Methods

Results

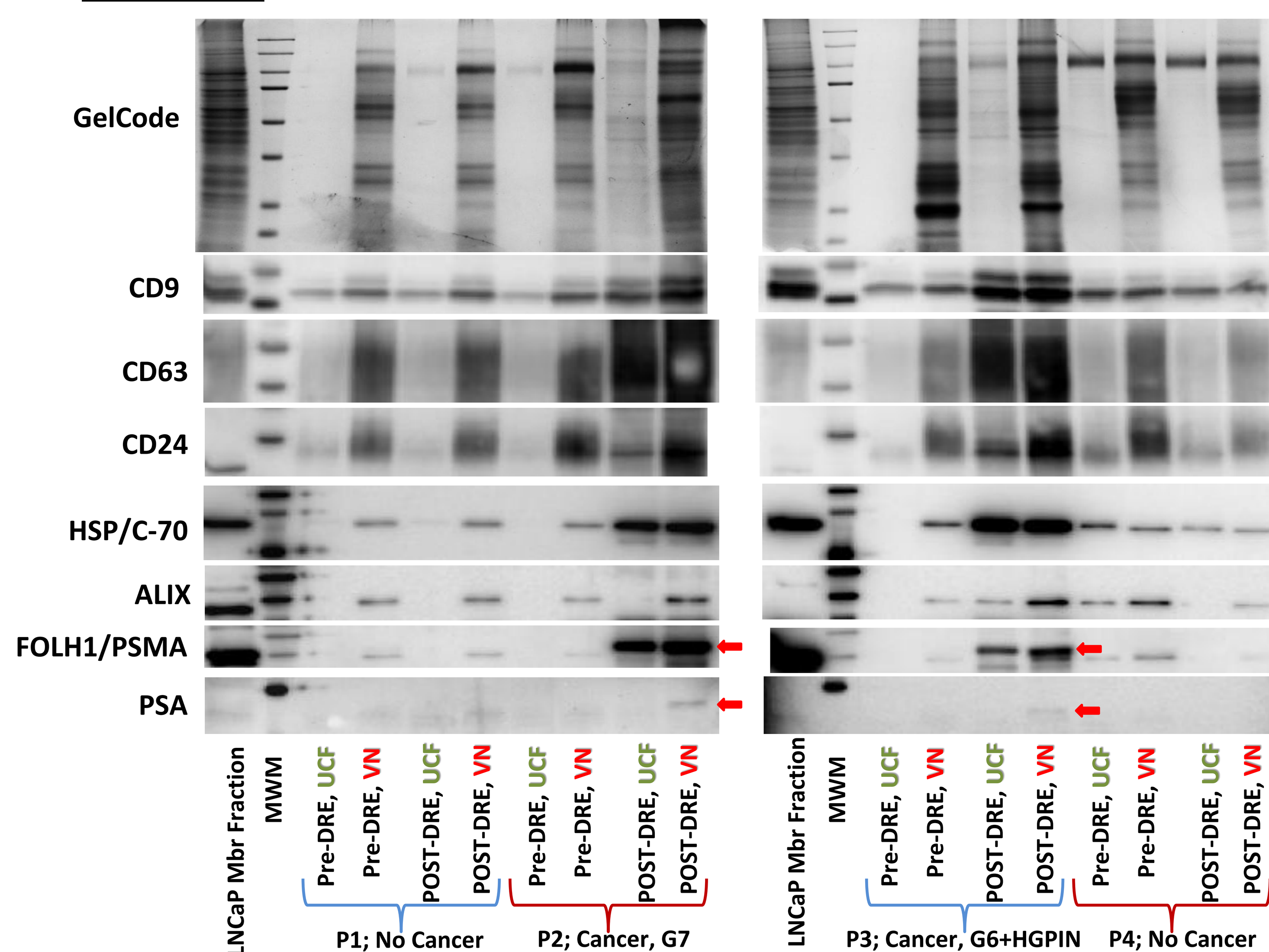

Figure 2. eMV pellets were isolated in parallel from equal volumes of pre- and post-DRE urines using ultracentrifugation and Vn peptide enrichment methods. Urinary Vn-eMV complexes contained canonical vesicle protein markers (CD9, CD63, CD24, HSP-70, ALIX), often in greater abundance than the corresponding UCF eMV fraction. Prostate-specific markers, PSMA and PSA, could be detected in post-DRE Vn urinary eMVs from PCa subjects.

UCF versus Vn96 Urinary eMV Enrichment Correlation of *PCA3/PSA* RT-qPCR Results

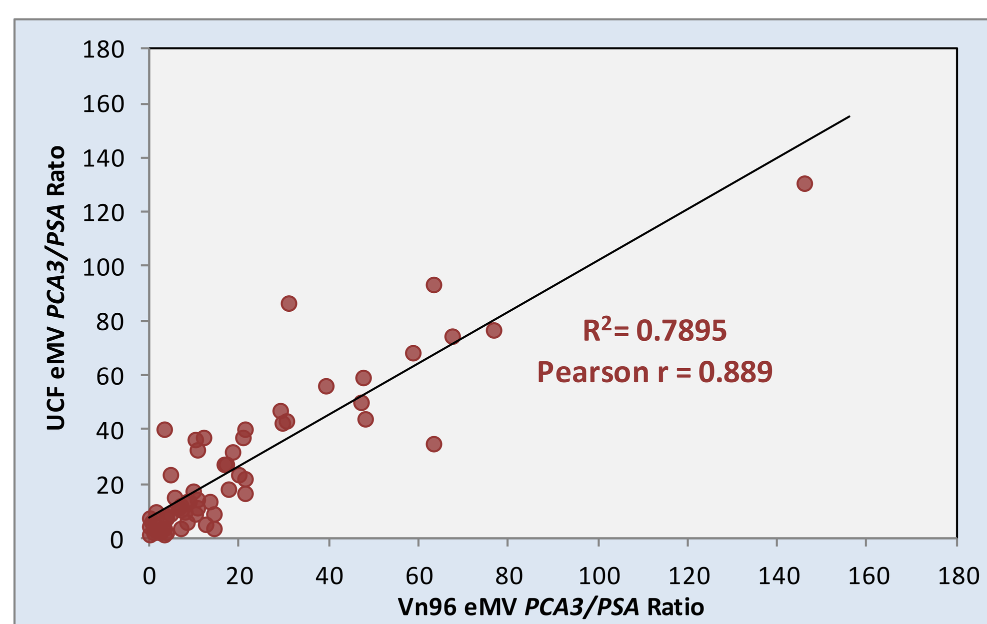

Figure 3. eMVs were isolated in parallel from equal volumes of post-DRE urines (N=68) using ultracentrifugation and Vn peptide enrichment methods. Total + small RNA was isolated from the resulting eMV pellets and transcripts for *PCA3* and *PSA* measured using RT-qPCR. *PCA3/PSA* ratios showed good agreement (Pearson r = 0.889) between UCF and Vn peptide eMVs.

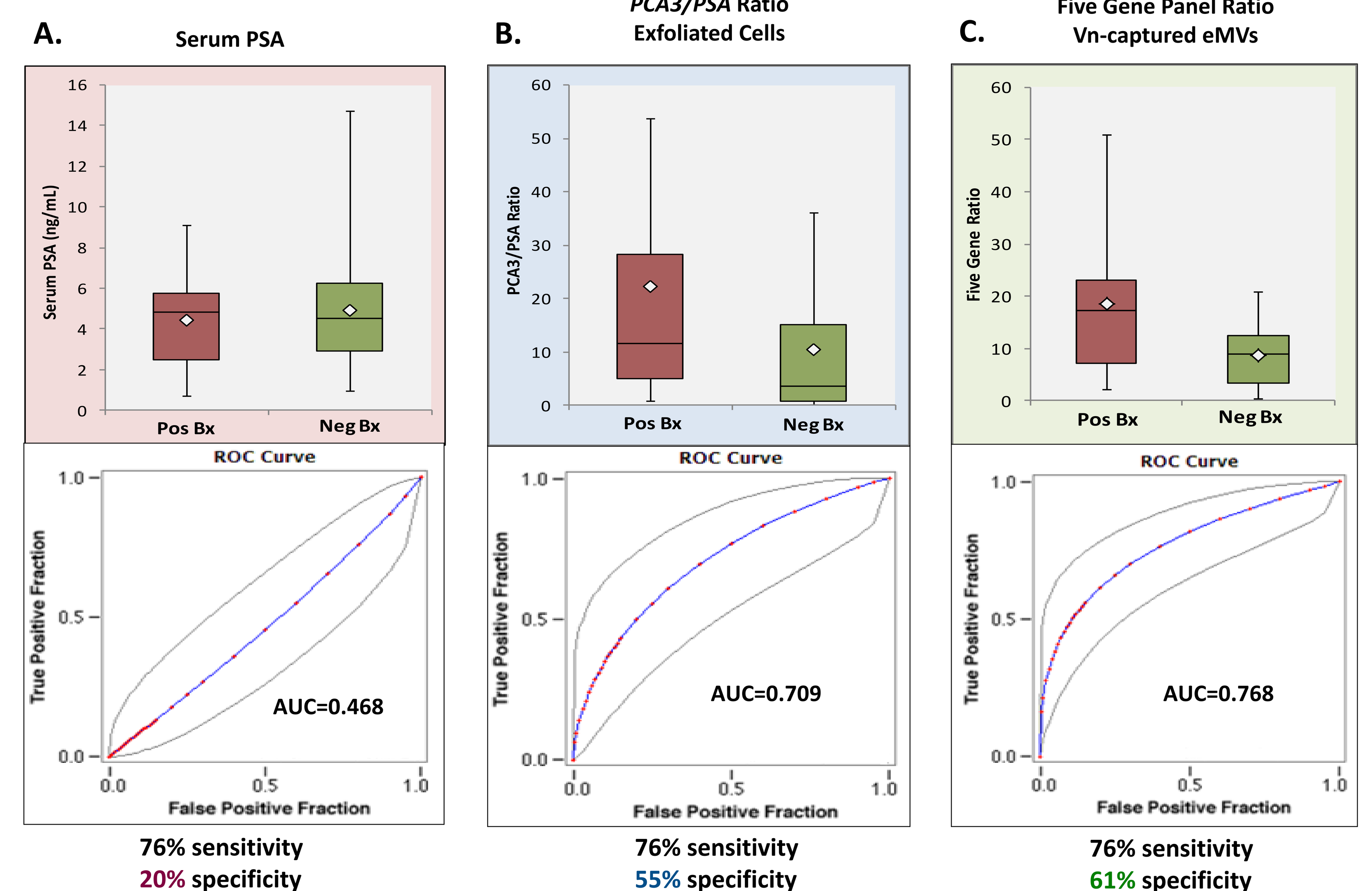

Figure 4. eMVs and exfoliated cells were isolated from post-DRE urine samples (Pos Bx N=32; Neg Bx N=36). Transcripts for the ACRI five gene panel and for *PCA3* and *PSA* were quantified by RT-qPCR. Serum PSA values were obtained from medical transcription reports. ROC curve analyses showed that ACRI's gene panel ratio (**C**), as measured using Vn eMV RNA, performed comparably to exfoliated cell *PCA3/PSA* ratio (**B**). Of note, was the somewhat better specificity of the ACRI gene ratio test compared to the *PCA3/PSA* ratio (61% versus 55%, respectively at a set sensitivity of 76%). The discriminatory power of the ACRI gene ratio was dramatically better than that of serum PSA (**A**), with an AUC for the gene panel of 0.768 compared to an AUC of 0.468 for serum PSA (a vast improvement in test specificity compared to serum PSA was noted).

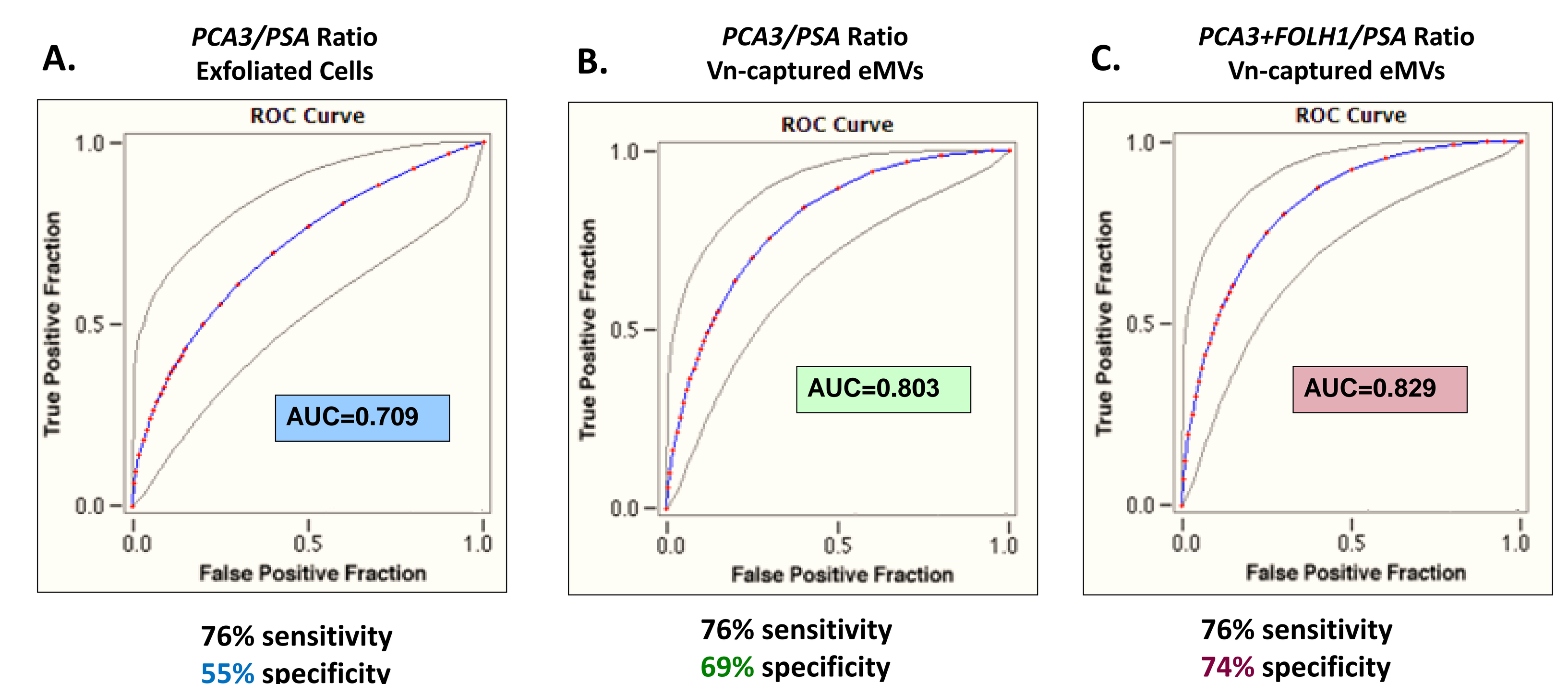

Figure 5. eMVs and exfoliated cells were isolated from post-DRE urine samples (Pos Bx N=32; Neg Bx N=36). Transcripts for *PCA3*, *PSA* and *FOLH1 (PSMA)* were quantified by RT-qPCR. ROC curve analyses showed an improvement in test accuracy and specificity when *PCA3/PSA* ratios were measured using Vn eMV RNA (**B**) compared to exfoliated cell RNA (**A**). The addition of FOLH1 measurements to the *PCA3/PSA* ratio (*PCA3+FOLH1/PSA*) further improved test specificity (**C**).

Conclusions

Vn96-captured urinary eMVs provide an enriched source of prostate-specific protein markers and diagnostically informative mRNA transcripts. Our five gene panel shows promising diagnostic potential as measured using Vn96 eMVs as the RNA source. A simple eMV enrichment method, as provided by our Vn96 peptide, is critical to the discovery and clinical application of PCa biomarkers in urine. ©

Relevant References

1. Cuperlovic-Culf M , Belacel N, Davey M and Ouellette RJ (2010) Multi-gene biomarker panel for reference free prostate cancer diagnosis: determination and independent validation. *Biomarkers* 15: 693-706.
2. Nilsson J, Skog J, Nordstrand A, et al (2009) Prostate cancer-derived urine exosomes : a novel approach to biomarkers for prostate cancer. *Br. J. Cancer* 100: 1603-1607.
3. Duijvesz D, Luider T, Bangma CH and Jenster G (2011) Exosomes as biomarker treasure chests for prostate cancer. *Eur Urol* 59:823-831.

Acknowledgements

ACRI is grateful for funding provided by the New Brunswick chapter of Canada's Ride for Dad through The Prostate Cancer Fight Foundation and funding provided by the Atlantic Canada Opportunities Agency (ACOA)