Introduction: Early and accurate detection of prostate cancer (PCa) is critical for positive patient outcomes. Of particular importance to PCa is the ability to determine the aggressiveness of a tumour with minimal invasiveness, a limitation of current biomarker-based exams. In this work, we propose a novel non-viral gene construct called tumour-activatable minicircles (TA-MCs), which are shortened versions of plasmids that are able to force cancer cells, but not normal cells, to produce a unique and sensitive biomarker. We initially designed our TA-MCs to express secreted embryonic alkaline phosphatase (SEAP), a reporter protein detectable in blood. Gene expression was mediated by the survivin promoter (pSurv), which is transcriptionally active only in cancers, ensuring SEAP is only produced when TA-MCs are delivered into cancer cells. These TA-MCs produced blood SEAP that was significantly higher in mice with aggressive tumours than mice with non-aggressive tumours. Showing promise as a platform for prostate cancer detection and characterization, we sought to expand the utility of our minicircle system by allowing for urine-based detection. Thus, we substituted SEAP on our TA-MCs with Gaussia luciferase (GLuc), a secreted reporter detectable in urine and potentially more sensitive than SEAP. Our objective was to build GLuc expressing TA-MCs and assess their ability to detect and characterize PCa across cell lines and in mice.

Materials and Methods: We first constructed parental plasmids, precursors to minicircles, that use pSurv to mediate expression of GLuc. Parental plasmids were then converted into TA-MCs using an established production system. We studied these TA-MCs in vitro via transfection of PCa cell lines of varying aggressiveness, measuring GLuc in cell supernatant using commercially available kits. Next, we assessed TA-MCs in nude mice with subcutaneous prostate tumours. TA-MCs complexed with polyethylenimine was injected intratumourally, then GLuc levels were measured in urine samples. Survivin expression was measured in cell and tumour lysates using Western Blot.

Results: We found that GLuc secretion was positively related to cellular survivin expression, being highest in PCa cells with the most survivin expression. Conversely, normal prostate cells secreted almost no GLuc. In mice with aggressive prostate tumours, previously undetectable GLuc was found in urine after TA-MCs administration.

Discussion: Our results thus far with GLuc are consistent with prior findings using SEAP as TA-MCs were able to produce GLuc according to survivin expression. Notably, urine GLuc can be successfully used to identify prostate tumour-bearing mice. The prospect of a convenient urine-based exam make TA-MCs powerful method to distinguish between patients with high-risk and indolent prostate tumours. Our next step is to compare GLuc in mice with tumours of different aggressiveness and explore TA-MCs as a potential agent for PCa theranostics.