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Presentation Title: **Development of a novel immunoassay for human kallikrein 14, a putative ovarian and breast cancer biomarker**

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Human kallikrein 14 (hK14; encoded by the *KLK14* gene) is a recently identified secreted serine protease, belonging to a family of potential cancer biomarkers which includes prostate-specific antigen (PSA). Our previous studies have demonstrated that the *KLK14* gene is steroid hormone-regulated, differentially expressed in breast, ovarian, prostate and testicular cancers and has prognostic value for breast and ovarian cancer patients at the mRNA level. We have recently discovered that hK14 protein levels are elevated in the serum of a proportion of breast and ovarian cancer patients and in cancerous ovarian tissues, suggesting that hK14 may have also diagnostic value. In order to further examine the potential clinical utility of hK14 as a cancer biomarker for endocrine-related malignancies, we have developed a novel, highly sensitive enzyme linked immunosorbent assay (ELISA). Recombinant hK14 was expressed in *P. pastoris* as the mature enzyme and purified to homogeneity from the culture supernatant after 6 days of methanol induction by cation exchange chromatography. Its identity was verified by tandem mass spectrometry. Purified recombinant hK14 was then used to generate polyclonal and monoclonal antibodies. A sandwich-type ELISA with a monoclonal-polyclonal antibody configuration was developed, coupled with a time-resolved fluorometric detection technique. The hK14-ELISA is sensitive (detection limit of 0.05 µg/L), specific for hK14 (<0.1% cross-reactivity with other kallikreins), and linear from 0.05 to 10 µg/L with between-run and within-run coefficients of variation of <10%. The ELISA was used to quantify hK14 levels with 36 normal tissue extracts, 9 biological fluids and conditioned media from 1 astrocytoma, 11 breast cancer, 1 cervical cancer, 1 colon cancer, 3 neuroblastoma, 2 osteosarcoma, 6 ovarian cancer, 1 pancreatic cancer and 8 prostatic cancer cell lines. The highest levels of hK14 were found in skin (245 ng hK14/g total protein), breast (224 ng hK14/g total protein) and prostatic (131 ng hK14/g total protein) tissue extracts. Among the cancer cell lines studied, hK14 was only found in the conditioned media of a subset of breast cancer (MCF7, MDA453), ovarian cancer (TOV21G, MDAH2774, OV-90, TOV112D), osteosarcoma (Saos) and neuroblastoma (SK-N-MC) cell lines. hK14 was also present in amniotic fluid (7.3±4.4µg/L), seminal plasma (6.3±3.1µg/L), saliva (4.4±2.1µg/L), follicular fluid (1.3±2.1µg/L), breast milk (1.2±0.4µg/L), ascites fluid from metastatic ovarian cancer patients (0.34±0.7µg/L), the serum of prostate cancer patients (0.3±0.9µg/L) and breast tumour cytosolic extracts (0.13±0.09µg/L), but not in cerebrospinal fluid. This novel ELISA will facilitate further studies on the role of hK14 as a cancer biomarker, which may ultimately aid in the diagnosis, prognosis and/or monitoring of ovarian, breast and prostate carcinoma.

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