EXECUTIVE SUMMARY

- Blood-based, early stage colorectal cancer (CRC) detection is a population-compliant unmet clinical need where confirmatory colonoscopy followed by surgically-actionable resection can be curative.

- Our technology offers a solution to currently-inadequate faecal occult blood early-stage CRC screening detection in the form of:
  - Immuno-ultradepletion methodology efficient in depleting the top 200 most abundant plasma proteins;
  - Cutting-edge, superb analytical specificity SWATH mass spectrometry (MS) analysis for biomarker discovery;
  - Blood-based multiplexed protein biomarker assay based on 6 novel markers for early stage screening of CRC;

- We are a multidisciplinary team of colorectal cancer clinicians and biologists with proteomics/mass spectrometry expertise and clinical biomarker and bioinformatics expertise, led by Professor Mark Baker at Macquarie University, who is Past-President of the Human Proteome Organisation (HUPO) and recently elected Chair of the Human Proteome Project. We have close ties with prominent CRC surgeons, pathologists and local hospital networks.

BACKGROUND

Colorectal cancer (CRC), also referred to as colon or bowel cancer, is the third most diagnosed cancer in the world (1.3M patients globally) causing one-third of cancer-related deaths. Overall survival rate is greatly influenced by the clinical stage of CRC when diagnosis occurs, with early stage CRC (if detected) potentially cured with surgery. However, due to the absence of symptoms or reluctance of populations to be involved in faecal screening programs, CRC is often discovered at late metastatic stages (~2/3 of patients). Current standard screening methods include faecal immunochemical test (FIT) or immunofaecal occult blood tests (iFOBT) as a precursor to colonoscopy if positive. FIT and iFOBT are linked to low patient compliance and poor sensitivity/specificity, especially for early stage CRC lesions that do not bleed into the bowel. Despite poor diagnostic statistics for FIT/iFOBT, follow-up of any positive result with extraordinarily accurate colonoscopy has saved lives. Detection of early stage lesions is critical, as their surgical removal will result in cure. Colonoscopy is invasive, requires sedation and bowel preparation and is often provided too late due to asymptomatic progression. Liquid biopsy tests measuring circulating tumour DNA (ctDNA) are at early stages of development in CRC but mostly are under consideration for clinical applications like detection of recurrence. Such tests, however, are yet to demonstrate sufficient sensitivity in clinical practice. Therefore, reliable early CRC detection remains a challenge and new solutions may result in a cure by surgery and possibly avoidance of unwarranted use of chemo- and radiotherapy. Blood-based screening tests are clearly preferable, however, none have yet been approved by the FDA/TGA or demonstrate sufficient diagnostic sensitivity and specificity.

PROTEOMIC-BASED DIAGNOSTICS

Protein biomarkers are already routinely utilised in the clinic using standard analytical techniques. Currently, there are over 20 FDA-approved/cleared protein biomarkers for a variety of oncology applications (e.g., PSA, CEA, CA125). Equally, OVA1 was the first FDA-cleared (2009) blood-based in vitro diagnostic multivariate index assay (IVDMIA) of proteomic biomarkers. In non-cancer applications, several proteomic-based MS tests have been approved by FDA in recent years (e.g. BioMérieux VITEK® MS and Bruker MALDI Biotyper CA System), which may have a significant impetus to future FDA approvals for MS-based diagnostics. Modern methods like SWATH-MS have been recognised to have exquisite analytical specificity for protein detection and identification, with clear opportunity to be used in clinical applications.

THE UNMET NEED

Despite significant advances and increasing acceptance of mass-spectrometry based diagnostics, there is a need for rapid, non-invasive, specific, sensitive, blood-based population-based screening tool for detection of early stage CRC, which would significantly minimise unwarranted procedures from high false positive FITs/iFOBTs, reduce disease burden, increase late stage detection and dramatically decrease mortality.
Biomarkers of Early-Stage Colorectal Cancer (enabled by ultradepletion technology)

OUR TECHNOLOGY

**Abundant Protein Ultradepletion:** The biggest limitation to detecting low abundance cancer biomarker proteins in plasma that play a critical role in assessing disease risk, response to treatments and indicate disease progression are that they are masked by very high abundance proteins in plasma. We have developed and optimised a cost-effective, rapid and efficient ultradepletion method that utilises chicken IgY polyclonal antibody-based columns against different ion-exchange fractions of human plasma to remove ~ 200 of the most abundant human plasma proteins, coupled with commercially available depletion columns. This method depletes almost all liver-synthesised acute phase response proteins that are often mis-identified as specific biomarkers of human cancer. The anti-human plasma IgY antibodies have been purified using a trade secret, commercial-scale, high-lipid yolk extraction methodology developed at Macquarie University and commercial quantity reserves of stored crude yolk IgY antibodies are available frozen at -70°C.

**MS-based Biomarker Discovery:** We have coupled IgY plasma ultradepletion technology with SWATH mass spectrometry (MS) to identify new early stage markers of CRC that could be used as the basis for an early-stage detection population-based screening test for CRC, prior to confirmatory colonoscopy and potentially curative surgery. SWATH-MS allows comprehensive capture and quantitation, high accuracy, sensitivity and specificity, excellent depth of coverage and re-analysis of data as libraries improve without re-acquisition.

Following ultradepletion, “pools” of blood samples from 20 patients with different stages of CRC (stages I-IV) or their respective controls were quantified using SWATH-MS using a strict statistical approach. From a pool of candidates, we have (for the first time) identified and confirmed using other immunological methods (Westerns, ELISA) six novel differentially-expressed lower abundance proteins (F2, HGFAC, PON1, CST3, ADAMDEC1 and CFD) that are biomarkers of early stage CRC (AJCC I/II). Our SWATH-MS study also confirmed previously discovered biomarkers, although sensitivity was not of the depth to identify reported biomarker candidates (e.g., suPAR, CEA, IL-8, CA19-9).

**Figure 1.** Employed early-stage CRC biomarker discovery methodology that couples immune-ultradepletion of high abundance proteins (~ top 200) and the use of SWATH mass spectrometry

**BIOMARKER INTENDED USE**

Population blood-based screening as an adjunct to follow-up colonoscopy to detect early-stage CRC
OFFICE OF COMMERCIALISATION AND INNOVATION

Biomarkers of Early-Stage Colorectal Cancer (enabled by ultradepletion technology)

RESEARCH EXPERTISE
A multidisciplinary team of excellent cancer biologists and clinicians with proteomics/mass spectrometry expertise, clinical biomarker and bioinformatics expertise led by Professor Mark S Baker. Mark is an internationally recognised proteomics cancer researcher, the Past-President of HUPO and current Chair of the global Human Proteome Project. Team members include noted CRC faecal biomarker and separation science expert Professor Edward Nice, and early career cancer researchers Dr Sadia Mahboob, Dr SB (Charlie) Ahn and Ms Samridhi Sharma. We have built our translational, clinical and CRC biobanking teams around access to clinical CRC patient samples through long-term fruitful collaborations with internationally-renowned CRC surgeon Professor Pierre Chapuis and cancer pathologist A/Professor Charles Chan, both from Concord Repatriation Hospital, University of Sydney. These clinical researchers co-developed guidelines for Australia’s CRC staging system and manage Australia’s premier CRC biobank and clinical database.

INTELLECTUAL PROPERTY
WO 2017/219093. Screening Methods
Prof Baker is an inventor on a family of granted patents: Depletion of Plasma Proteins
Significant undisclosed know-how

PUBLICATIONS


PARTNERSHIP OPPORTUNITY
We are actively pursuing clinical biomarker validation and assay development in collaboration with colorectal surgeons. We are looking for a strategic partnership with the diagnostic or proteomic tools industry to further develop this product or to utilise the ultradepletion/SWATH technology for other blood-based biomarker discovery.

LIKE TO KNOW MORE?
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References
1 Siegel et al (2014) CA: Cancer J Clin 64 (2) 104-117;