

Cancer Biomarker Research Literatures

Ma Hongbao ¹, Margaret Ma ², Yang Yan ¹

¹ Brookdale Hospital, Brooklyn, New York 11212, USA; ² Cambridge, MA 02138, USA
ma8080@gmail.com

Abstract: Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries. This article introduces recent research reports as references in the related studies. This article introduces recent research reports as references in the cancer biomarkers related studies.

[Ma H, Young M, Yang Y. **Cancer Biomarker Research Literatures**. Cancer Biology 2015;5(2):96-110]. (ISSN: 2150-1041). <http://www.cancerbio.net>. 9

Key words: cancer; biomarker; life; research; literature

1. Introduction

Cancer is the general name for a group of more than 100 diseases. Although there are many kinds of cancer, all cancers start because abnormal cells grow out of control. Untreated cancers can cause serious illness and death. The body is made up of trillions of living cells. Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide faster to allow the person to grow. After the person becomes an adult, most cells divide only to replace worn-out or dying cells or to repair injuries.

The following introduces recent reports as references in the related studies.

Alisoltani, A., H. Fallahi, et al. "RNA-seq SSRs and small RNA-seq SSRs: new approaches in cancer biomarker discovery." *Gene*. 2015 Apr 10;560(1):34-43. doi: 10.1016/j.gene.2015.01.027. Epub 2015 Jan 30.

The recent exponential increase in the number of next generation sequencing studies provides a new source of data for the discovery of functional genomics based markers. The RNA-seq and small RNA-seq provide a new source for the discovery of differentially expressed SSRs (simple sequence repeats) as biomarkers in various diseases. In the present study, for the first time, we applied RNA-seq SSR to find new biomarkers for pancreatic cancer (PC) diagnosis. Analysis of RNA-seq data revealed a significant alternation in the frequency of SSR motifs during cancer progression. In particular, RNA-seq SSR showed an increase in the frequencies of GCC/GGC and GCG/CGC motifs in PC samples compared to healthy pancreas. These findings were further confirmed using meta-analysis of EST-SSR

data in 11 different cancers. Interestingly, the genes containing GCC/GGC and GCG/CGC motifs in their sequences were involved in many cancer-related biological processes, particularly regulation processes. The small RNA-seq data were also mined for the conserved patterns in SSR frequencies (sRNA-seq SSR) during cancer progression. Based on the results, we suggest the potential use of GCC/GGC and GCG/CGC motifs as biomarkers in PC. Based on the findings of this study, it seems that RNA-seq SSR and sRNA-seq SSR could open a new paradigm in the diagnostic and even therapeutic strategies for PC along the other types of cancers.

Cao, G., G. Hajisalem, et al. "Quantification of an exogenous cancer biomarker in urinalysis by Raman spectroscopy." *Analyst*. 2014 Nov 7;139(21):5375-8. doi: 10.1039/c4an01309c.

We quantified an exogenous cancer biomarker, Acetyl amantadine (AcAm), directly from urine solution using surface enhanced Raman spectroscopy (SERS). SERS was used for the detection of AcAm using a commercial Raman substrate after beta-cyclodextrin encapsulation for capture of the analyte. We achieved a detection limit of 1 ng mL⁻¹ of AcAm in the mock urine in the absence of steroids without extraction or other pre-treatment methods required. With levels of corticosterone typical of urine, the limit of detection was 30 times higher. Since the approach works directly from samples containing the high concentrations of salts and organic co-solutes normal to urine, it has the potential to reduce cost and speed up processing with respect to methods that require pre-purification. Therefore, this is promising for clinical adoption for early cancer detection, particularly for lung cancer.

Chambers, A. G., A. J. Percy, et al. "MRM for the verification of cancer biomarker proteins: recent applications to human plasma and serum." Expert Rev Proteomics. 2014 Apr;11(2):137-48. doi: 10.1586/14789450.2014.877346. Epub 2014 Jan 29.

Accurate cancer biomarkers are needed for early detection, disease classification, prediction of therapeutic response and monitoring treatment. While there appears to be no shortage of candidate biomarker proteins, a major bottleneck in the biomarker pipeline continues to be their verification by enzyme linked immunosorbent assays. Multiple reaction monitoring (MRM), also known as selected reaction monitoring, is a targeted mass spectrometry approach to protein quantitation and is emerging to bridge the gap between biomarker discovery and clinical validation. Highly multiplexed MRM assays are readily configured and enable simultaneous verification of large numbers of candidates facilitating the development of biomarker panels which can increase specificity. This review focuses on recent applications of MRM to the analysis of plasma and serum from cancer patients for biomarker verification. The current status of this approach is discussed along with future directions for targeted mass spectrometry in clinical biomarker validation.

Delgado, A. P., S. Hamid, et al. "A novel transmembrane glycoprotein cancer biomarker present in the X chromosome." Cancer Genomics Proteomics. 2014 Mar-Apr;11(2):81-92.

BACKGROUND: The uncharacterized proteins of the human proteome offer an untapped potential for cancer biomarker discovery. Numerous predicted open reading frames (ORFs) are present in diverse chromosomes. The mRNA and protein expression data, as well as the mutational and variant information for these ORF proteins are available in the cancer-related bioinformatics databases. **MATERIALS AND METHODS:** ORF proteins were mined using bioinformatics and proteomic tools to predict motifs and domains, and cancer relevance was established using cancer genome, transcriptome and proteome analysis tools. **RESULTS:** A novel testis-restricted ORF protein present in chromosome X called CXorf66 was detected in the serum, plasma and neutrophils. This gene is termed secreted glycoprotein in chromosome X (SGPX). The SGPX gene is up-regulated in cancer of the brain, lung and in leukemia, and down-regulated in liver and prostate cancer. Brain cancer in female patients exhibited elevated copy numbers of the SGPX gene. **CONCLUSION:** The SGPX gene is a putative novel cancer biomarker. Our results demonstrate the feasibility of mining the 'dark

matter' of the cancer proteome for rapid cancer biomarker discovery.

Derks, S., A. H. Cleven, et al. "Emerging evidence for CHFR as a cancer biomarker: from tumor biology to precision medicine." Cancer Metastasis Rev. 2014 Mar;33(1):161-71. doi: 10.1007/s10555-013-9462-4.

Novel insights in the biology of cancer have switched the paradigm of a "one-size-fits-all" cancer treatment to an individualized biology-driven treatment approach. In recent years, a diversity of biomarkers and targeted therapies has been discovered. Although these examples accentuate the promise of personalized cancer treatment, for most cancers and cancer subgroups no biomarkers and effective targeted therapy are available. The great majority of patients still receive unselected standard therapies with no use of their individual molecular characteristics. Better knowledge about the underlying tumor biology will lead the way toward personalized cancer treatment. In this review, we summarize the evidence for a promising cancer biomarker: checkpoint with forkhead and ring finger domains (CHFR). CHFR is a mitotic checkpoint and tumor suppressor gene, which is inactivated in a diverse group of solid malignancies, mostly by promoter CpG island methylation. CHFR inactivation has shown to be an indicator of poor prognosis and sensitivity to taxane-based chemotherapy. Here we summarize the current knowledge of altered CHFR expression in cancer, the impact on tumor biology and implications for personalized cancer treatment.

Devi, R. V., M. Doble, et al. "Nanomaterials for early detection of cancer biomarker with special emphasis on gold nanoparticles in immunoassays/sensors." Biosens Bioelectron. 2015 Jun 15;68:688-98. doi: 10.1016/j.bios.2015.01.066. Epub 2015 Jan 29.

At the onset of cancer a selective protein or gene based biomarker gets elevated or modified in body fluids or tissues. Early diagnosis of these markers can greatly improve the survival rate or facilitate effective treatment with different modalities. Though the sophisticated imaging technologies like Magnetic Resonance Imaging, Positron Emission Tomography and Computed Tomography have the impact of nanotechnology on their improved performance, they are however unsuitable for early detection of cancer biomarkers or their quantification. Other approaches for cancer diagnosis based on cell morphology and microscopy (biopsies) are too not conclusive for early diagnosis of cancer. The only hope for early diagnosis of cancer in near future is by the detection of cancer biomarkers using immunoassays/sensors that are reformed by Nanotechnology. Attractive properties of nanoparticles have miraculously lifted up the design,

fabrication, sensitivity and multiplexing of these immunoassays/sensors in biomarker detection. With this aspect we have explored the recent advancements in immunosensing techniques that were developed exploiting the unique properties of gold nanoparticles. We have also discussed the possible future trends with respect to gold nanoparticle-coupled microfluidic sensors; paper based analytical devices and the single-molecule biosensing.

Eaton, R. M., J. A. Shallcross, et al. "Selection of DNA aptamers for ovarian cancer biomarker HE4 using CE-SELEX and high-throughput sequencing." Anal Bioanal Chem. 2015 Apr 12.

The development of novel affinity probes for cancer biomarkers may enable powerful improvements in analytical methods for detecting and treating cancer. In this report, we describe our use of capillary electrophoresis (CE) as the separation mechanism in the process of selecting DNA aptamers with affinity for the ovarian cancer biomarker HE4. Rather than the conventional use of cloning and sequencing as the last step in the aptamer selection process, we used high-throughput sequencing on an Illumina platform. This data-rich approach, combined with a bioinformatics pipeline based on freely available computational tools, enabled the entirety of the selection process-and not only its endpoint-to be characterized. Affinity probe CE and fluorescence anisotropy assays demonstrate the binding affinity of a set of aptamer candidates identified through this bioinformatics approach.

Egleton, J. E., C. C. Thinnis, et al. "Structure-activity relationships and colorimetric properties of specific probes for the putative cancer biomarker human arylamine N-acetyltransferase 1." Bioorg Med Chem. 2014 Jun 1;22(11):3030-54. doi: 10.1016/j.bmc.2014.03.015. Epub 2014 Mar 28.

A naphthoquinone inhibitor of human arylamine N-acetyltransferase 1 (hNAT1), a potential cancer biomarker and therapeutic target, has been reported which undergoes a distinctive concomitant color change from red to blue upon binding to the enzyme. Here we describe the use of in silico modeling alongside structure-activity relationship studies to advance the hit compound towards a potential probe to quantify hNAT1 levels in tissues. Derivatives with both a fifty-fold higher potency against hNAT1 and a two-fold greater absorption coefficient compared to the initial hit have been synthesized; these compounds retain specificity for hNAT1 and its murine homologue mNat2 over the isoenzyme hNAT2. A relationship between pKa, inhibitor potency and colorimetric properties has also been uncovered. The high potency of representative examples against hNAT1 in ZR-75-1 cell extracts also

paves the way for the development of inhibitors with improved intrinsic sensitivity which could enable detection of hNAT1 in tissue samples and potentially act as tools for elucidating the unknown role hNAT1 plays in ER+ breast cancer; this could in turn lead to a therapeutic use for such inhibitors.

Eletxigerra, U., J. Martinez-Perdiguero, et al. "Amperometric magnetoimmunosensor for ErbB2 breast cancer biomarker determination in human serum, cell lysates and intact breast cancer cells." Biosens Bioelectron. 2015 Aug 15;70:34-41. doi: 10.1016/j.bios.2015.03.017. Epub 2015 Mar 10.

A highly sensitive amperometric magnetoimmunosensor for the determination of ErbB2 protein, a well-known biomarker related to high-impact high-incidence diseases such as breast cancer, is described. A sandwich format involving the covalent immobilization of a specific capture antibody onto magnetic beads (MBs) and incubation of the modified MBs with a mixture solution of the antigen and a HRP-labeled detector antibody was used. The resulting modified MBs were captured on the surface of a disposable screen-printed carbon electrode (SPCE) and the amperometric responses at -0.20V were measured. This ErbB2 magnetoimmunosensor exhibited a very low detection limit of 26pgmL(-)(1) far below the established cut-off for this biomarker (15ngmL(-1)) and was successfully applied to the quantitation of ErbB2 in human serum and cell lysates samples without any matrix effect. In addition, the developed assay allowed the assessment of ErbB2 status directly in intact breast cancer cells. The results correlated well with those obtained with a commercial ELISA method, thus demonstrating that the new magnetoimmunosensing platform offers a truthful and useful analytical tool to be easily applied in breast cancer diagnosis through either ErbB2 protein determination or breast cancer cell status detection.

Emami, M., M. Shamsipur, et al. "An electrochemical immunosensor for detection of a breast cancer biomarker based on antiHER2-iron oxide nanoparticle bioconjugates." Analyst. 2014 Jun 7;139(11):2858-66. doi: 10.1039/c4an00183d.

A label free immunosensor was designed for ultra-detection of human epidermal growth factor receptor 2 (HER2) in real samples using a differential pulse voltammetry (DPV) method. In a separate process, antiHER2 antibodies were attached to iron oxide nanoparticles (Fe3O4 NPs) to form stable bioconjugates which were later laid over the gold electrode surface. In this way, by the advantage of their long terminals, the bioconjugates provided the most possible space for the immuno-reaction between biomolecules. Under optimal conditions, the

immunosensor was responsive to HER2 concentrations over the ranges of 0.01-10 ng mL⁻¹ and 10-100 ng mL⁻¹ linearly and benefited from a satisfactory detection limit as low as 0.995 pg mL⁻¹ and a favorable sensitivity as sharp as 5.921 μ A mL ng⁻¹. The reliability of the method in clinical analysis was proved by successful quantization of HER2 levels in serum samples obtained from patients. Furthermore, the precision and the stability of the method were evaluated and verified to be acceptable in immunoassay studies.

Espinoza-Castaneda, M., A. de la Escosura-Muniz, et al. "Nanochannel array device operating through Prussian blue nanoparticles for sensitive label-free immunodetection of a cancer biomarker." *Biosens Bioelectron.* 2015 May 15;67:107-14. doi: 10.1016/j.bios.2014.07.039. Epub 2014 Jul 24.

A novel nanochannel array (NC) device that operates through Prussian blue nanoparticles (PBNPs) as redox indicator for sensitive label free immunodetection of a cancer biomarker is presented. Stable and narrow-sized (around 4 nm) PBNPs, protected by polyvinylpyrrolidone, exhibited a well-defined and reproducible redox behavior and were successfully applied for the voltammetric evaluation of the nanochannels (20 nm pore sized) blockage due to the immunocomplex formation. The bigger size of the PBNPs compared with ionic indicators such as the [Fe(CN)₆]^{4-/3-} system leads to an increase in the steric effects hindering their diffusion toward the signaling electrode which in turn is transduced to an improvement of the detection limit from 200 μ g mL⁻¹ to 34 pg human IgG mL⁻¹. This novel and effective PBNPs-NC technology for the detection of small proteins captured inside the nanochannels is successfully applied for the quantification of a cancer biomarker (parathyroid hormone-related protein, PTHrP) in a real clinical scenario such as cell culture medium. The achieved label-free detection of PTHrP at levels of 50 ng mL⁻¹ is with great interest to study relevant functions that this protein exerts in normal tissues and cancer.

Gogalic, S., U. Sauer, et al. "Bladder cancer biomarker array to detect aberrant levels of proteins in urine." *Analyst.* 2015 Feb 7;140(3):724-35. doi: 10.1039/c4an01432d.

Bladder cancer (BCa) is a serious malignancy of the urinary tract worldwide and also prominent for its high rate of recurrence incorporating 50% of all treated patients. To reduce relapse of BCa, lifelong surveillance of patients is essential leading to high treatment costs. The gold standard for the diagnosis of bladder cancer is cystoscopy. It is very sensitive, but due to high costs and its invasive nature this method

for routine diagnosis of bladder cancer remains questionable. Because of this and the required surveillance of patients suffering from bladder cancer, urine based markers represent a new potential field of investigation. Literature at the National Center of Biological Information (NCBI) was retrieved for a potential marker panel offering specific protein signatures and used to develop a sensitive and accurate chip assay to monitor BCa. Discovery of possible bladder cancer protein markers is compiled by extensive literature search including 1077 recently (15.01.2008-20.03.2014) published research articles. Validation of this literature is done by selection based on prior defined inclusion and exclusion criteria. A set of six putative biomarkers (VEGF, IL-8, MMP-9, MMP-7, survivin and Cyfra 21.1) was identified and a non-invasive microarray developed to be used for further clinical validation. Investigation regarding optimized urine preparation and assay development, to enhance assay sensitivity for the marker panel, was carried out. This protein based BCa chip enables the fast (within 5 h), simultaneous, easy to operate, cheap, early and non-invasive determination of BCa and is ready for clinical evaluation.

Hadad, S. M., P. Coates, et al. "Evidence for biological effects of metformin in operable breast cancer: biomarker analysis in a pre-operative window of opportunity randomized trial." *Breast Cancer Res Treat.* 2015 Feb;150(1):149-55. doi: 10.1007/s10549-015-3307-5. Epub 2015 Feb 15.

Metformin has therapeutic potential against breast cancer, but the mechanisms of action in vivo remain uncertain. This study examined biomarker effects of metformin in primary breast cancer in a preoperative window of opportunity trial. Non-diabetic women with operable invasive breast cancer were randomized to receive open label pre-operative metformin (500 mg daily for 1 week then 1 g twice daily for a further week) or as controls, not receiving metformin. Patients in both arms had a core biopsy pre-randomisation and again at the time of surgery. Immunohistochemistry for phospho-AMPK (pAMPK), phospho-Akt (pAkt), insulin receptor, cleaved caspase-3, and Ki67 was performed on formalin-fixed paraffin-embedded cores, scored blinded to treatment and analysed by paired t test. In metformin-treated patients, significant up-regulation of pAMPK (paired t test, $p = 0.04$) and down-regulation of pAkt (paired t test, $p = 0.043$) were demonstrated compared to the control group. Insulin receptor and serum insulin remained similar following metformin treatment compared with a rise in insulin receptor and insulin in controls. Significant falls in Ki67 and cleaved caspase-3 (paired t test, $p = 0.044$) were seen in the metformin-treated patients but not in the control

group. Changes were independent of body mass index. These biomarker data suggest mechanisms for metformin action in vivo in breast cancer patients via up-regulation of tumor pAMPK, down-regulation of pAkt, and suppression of insulin responses reflecting cytostatic rather than cytotoxic mechanisms.

Haldrup, C., N. Kosaka, et al. "Profiling of circulating microRNAs for prostate cancer biomarker discovery." *Drug Deliv Transl Res.* 2014 Feb;4(1):19-30. doi: [10.1007/s13346-013-0169-4](https://doi.org/10.1007/s13346-013-0169-4).

Prostate cancer (PC) is the most frequent cancer in men in the Western world. Currently, serum prostate-specific antigen levels and digital rectal examinations are used to indicate the need for diagnostic prostate biopsy, but lack in specificity and sensitivity. Thus, many men undergo unnecessary biopsy, and better and less invasive tools for PC detection are needed. Furthermore, whereas aggressive PC should be treated immediately to prevent dissemination, indolent PC often does not progress and overtreatment should be avoided. Currently, the best predictors of aggressiveness are Gleason score and T-stage of the primary PC. Better tools to assess PC aggressiveness could aid in treatment decisions. Recently, circulating miRNAs have been suggested as potential new biomarkers for PC with diagnostic and prognostic potential. Here, to identify new serum miRNA biomarker candidates for PC, we performed genome-wide miRNA profiling of serum samples from 13 benign prostatic hyperplasia (BPH) control patients and 31 PC patients. Furthermore, we carefully reviewed the literature on circulating miRNA biomarkers for PC. Our results confirmed the down-regulation of miR-141 and miR-375, two of the most well-documented candidate miRNA markers for PC. Moreover, we identified several new potential serum miRNA markers for PC and developed three novel and highly specific (100 %) miRNA candidate marker panels able to identify 84 % of all PC patients (miR-562/miR-210/miR-501-3p/miR-375/miR-551b), 80 % of patients with disseminated PC when compared to BPH patients (let-7a*/miR-210/miR-562/miR-616), and 75 % of disseminated PC patients when compared to localized PC patients (miR-375/miR-708/miR-1203/miR-200a), demonstrating high potential of serum miRNAs for diagnosing and staging of PC.

Hamelin-Peyron, C., V. Vlaeminck-Guillem, et al. "Prostate cancer biomarker annexin A3 detected in urines obtained following digital rectal examination presents antigenic variability." *Clin Biochem.* 2014 Jul;47(10-11):901-8. doi: [10.1016/j.clinbiochem.2014.05.063](https://doi.org/10.1016/j.clinbiochem.2014.05.063). Epub 2014 Jun 2.

OBJECTIVES: Annexin A3 (ANXA3) is a potential marker for prostate cancer (PCa). We aimed

to develop robust immunoassays suitable for quantifying ANXA3 in urine samples obtained following digital rectal examination (DRE) in order to facilitate the diagnostic performance evaluation of this marker. DESIGN AND METHODS: Anti-ANXA3 monoclonal antibodies were generated and their epitopes mapped. Two different ANXA3 assay prototypes were established on the VIDAS(R) automated immunoanalyser and analytical validation was carried out using post-DRE urine samples obtained from patients with PCa (n=23) or benign prostate hyperplasia (n=31). RESULTS: The assays had the same capture antibody (TGC44) but different detection antibodies (13A12 or 5C5), recognizing novel distinct epitopes. Both had a lower limit of quantification <1ng/mL and were highly specific for ANXA3, not cross-reacting with other annexins. Interassay imprecision was <=11% and <=15% for 13A12 and 5C5 assays, respectively. Surprisingly, a total lack of correlation was observed between ANXA3 levels measured by these two assays in post-DRE urines, indicating detection of distinct antigenic variants. Two freeze-thaw cycles did not affect analyte stability in either assay, whereas a lack of stability of antigenic variants was observed when samples were stored at -80 degrees C for 1month. CONCLUSIONS: Two different antigenic variants of ANXA3 are present in post-DRE urines and their clinical significance for diagnosis of prostate cancer should be further investigated. These variants are not stable over time in samples preserved at -80 degrees C. Until this issue is resolved, ANXA3 should only be measured in freshly collected samples.

Henrique, R. and C. Jeronimo "DNA hypomethylation in plasma as a cancer biomarker: when less is more?" *Expert Rev Mol Diagn.* 2014 May;14(4):419-22. doi: [10.1586/14737159.2014.905203](https://doi.org/10.1586/14737159.2014.905203). Epub 2014 Apr 1.

Early cancer detection strategies are required to identify patients at initial stages, when curative-intended treatment is more effective. Assessment of genome-wide hypomethylation might allow for early cancer detection in bodily fluids, but requires high throughput technologies, such as next generation sequencing. In the study under evaluation, performance of a hypomethylation and copy number aberration (CNA) assay for detection of hepatocellular carcinoma (HCC), based on massive parallel bisulfite sequencing of plasma DNA was assessed. Sensitive (92/69%) and specific (88/94%) HCC detection (using 'OR'/'AND' algorithms) was achieved using a mean sequencing depth of 93 million reads in one lane. Using the 'AND' or the 'OR' algorithms, other cancer types were detected with 60% sensitivity and 94% specificity, or 85% sensitivity and 88% specificity, respectively. Lowering sequencing depth to 10 million

reads (decreasing analysis time and cost) did not alter hypomethylation performance but impaired that of CNA.

Kadimisetty, K., S. Malla, et al. "Automated multiplexed ECL Immunoarrays for cancer biomarker proteins." *Anal Chem.* 2015 Apr 21;87(8):4472-8. doi: 10.1021/acs.analchem.5b00421. Epub 2015 Apr 9.

Point-of-care diagnostics based on multiplexed protein measurements face challenges of simple, automated, low-cost, and high-throughput operation with high sensitivity. Herein, we describe an automated, microprocessor-controlled microfluidic immunoarray for simultaneous multiplexed detection of small protein panels in complex samples. A microfluidic sample/reagent delivery cassette was coupled to a 30-microwell detection array to achieve sensitive detection of four prostate cancer biomarker proteins in serum. The proteins are prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), platelet factor-4 (PF-4), and interleukin-6 (IL-6). The six channel system is driven by integrated micropumps controlled by an inexpensive programmable microprocessor. The reagent delivery cassette and detection array feature channels made by precision-cut 0.8 mm silicone gaskets. Single-wall carbon nanotube forests were grown in printed microwells on a pyrolytic graphite detection chip and decorated with capture antibodies. The detection chip is housed in a machined microfluidic chamber with a steel metal shim counter electrode and Ag/AgCl reference electrode for electrochemiluminescent (ECL) measurements. The preloaded sample/reagent cassette automatically delivers samples, wash buffers, and ECL RuBPY-silica-antibody detection nanoparticles sequentially. An onboard microcontroller controls micropumps and reagent flow to the detection chamber according to a preset program. Detection employs tripropylamine, a sacrificial reductant, while applying 0.95 V vs Ag/AgCl. Resulting ECL light was measured by a CCD camera. Ultralow detection limits of 10-100 fg mL⁻¹ were achieved in simultaneous detection of the four protein in 36 min assays. Results for the four proteins in prostate cancer patient serum gave excellent correlation with those from single-protein ELISA.

Kamal, A. H., S. Power, et al. "Optimizing the quality of breast cancer biomarker use at Duke Cancer Institute." *J Natl Compr Canc Netw.* 2014 Feb;12 Suppl 1:S21-4.

Advances in identifying biomarker profiles in patients with early-stage breast cancer have improved 5-year curative rates. Identification of the HER2 receptor provides valuable information that has been

shown to extend survival in adjuvant and metastatic settings. Current clinical guidelines discuss when confirmatory testing may be inappropriate. Using a quality improvement approach, the team at Duke Cancer Institute determined HER2 ordering practices in a large academic cancer center. HER2 ordering using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) was abstracted from the charts of 314 patients with early-stage breast cancer. Qualitative responses to current clinical practices were obtained from clinicians. Of the patients included, duplicate IHC was performed for 36% and in triplicate for 6%; repeat testing resulted in clinically significant change in HER2 status for approximately 20%. Repeat biomarker testing on metastatic biopsy sites "all of the time" was favored by the surveyed physicians. FISH was ordered for each grade of IHC: 0+ (>20% of cases), 1+ (>20%), 2+ (99%), 3+ (54%). Most physicians "strongly" or "somewhat" favored solutions that integrate order sets and care pathways into the electronic medical record. This quality improvement project identified root causes and solutions to practice variance in breast cancer biomarker ordering and interpretation. Further investigations are planned to standardize best practices while appreciating the clinical challenges posed by discordant test results.

Kumar, S., A. Nag, et al. "A Comprehensive Review on miR-200c, a Promising Cancer Biomarker with Therapeutic Potential." *Curr Drug Targets.* 2015 Mar 25.

MicroRNAs (miRNAs) are small single stranded non coding RNA molecules (~22 nucleotides) which impede protein production by directly interacting with 3'untranslated regions of the target mRNAs. Interestingly, miR-200c is often dysregulated in various cancers that normally exhibits tumor suppressive behavior by blocking epithelial to mesenchymal transition (EMT) of cancer cells. However, elevation of miR-200c in various cancer tissues contradicts the tumor suppressive role of this microRNA. This review addresses the molecular mechanisms involved in the regulation of the endogenous level of miR-200c in various cancers such as breast, ovarian, prostate, endometrial, lungs, colon, pancreatic, etc. and its differential role in regulation of proliferation and EMT phenotype of cancer cells. Further, this review discusses whether abnormal level of miR-200c in cancer tissues or in blood circulation can be used as a biomarker. Importantly, how the level of miR-200c can be used to predict the effectiveness of the cancer therapy is also discussed. Accumulating evidences suggest that use of miR-200c alone may not be sufficient for treatment of cancer patients, but the combination of miR-200c with an anti-proliferating drug could be a better choice to prevent invasiveness

of cancers as well as tumor growth both in primary and in metastatic sites. This article also proposes that the tumor microenvironment may have a role in influencing epigenetic silencing of miR-200c expression.

Lastraioli, E., J. Iorio, et al. Ion channel expression as promising cancer biomarker, *Biochim Biophys Acta*. 2014 Dec 24. pii: S0005-2736(14)00452-0. doi: 10.1016/j.bbame.2014.12.016.

Cancer is a disease with marked heterogeneity in both response to therapy and survival. Clinical and histopathological characteristics have long determined prognosis and therapy. The introduction of molecular diagnostics has heralded an explosion in new prognostic factors. Overall, histopathology, immunohistochemistry and molecular biology techniques have described important new prognostic subgroups in the different cancer categories. Ion channels and transporters (ICT) are a new class of membrane proteins which are aberrantly expressed in several types of human cancers. Besides regulating different aspect of cancer cell behavior, ICT can now represent novel cancer biomarkers. A summary of the data obtained so far and relative to breast, prostate, lung, colorectal, esophagus, pancreatic and gastric cancers are reported. Special emphasis is given to those studies aimed at relating specific ICT or a peculiar ICT profile with current diagnostic methods. Overall, we are close to exploit ICTs for diagnostic, prognostic or predictive purposes in cancer. This article is part of a Special Issue entitled: Membrane channels and transporters in cancers.

Lund, F., P. H. Petersen, et al. "Criteria to interpret cancer biomarker increments crossing the recommended cut-off compared in a simulation model focusing on false positive signals and tumour detection time." *Clin Chim Acta*. 2014 Apr 20;431:192-7. doi: 10.1016/j.cca.2014.01.013. Epub 2014 Feb 6.

BACKGROUND: Several criteria have been proposed to interpret increments in serological cancer biomarker concentrations starting from low baseline concentrations crossing the cut-off. None of the criteria have been compared for their ability to signal tumour growth when $\leq 2\%$ false positive results are accepted. **METHODS:** The cancer biomarker Tissue Polypeptide Antigen was used as an example. Seven criteria to interpret increments in concentrations were investigated by computer simulations. Firstly, for each criterion, we identified a baseline concentration stratified for three levels of biological variation providing $\leq 2\%$ false positive signals of tumour growth during one year of monitoring. Secondly, combining the steady state concentrations with rates of marker increase during tumour growth allowed

calculation of the lengths of tumour detection times for each criterion. **RESULTS:** The number of false positive marker signals depended on the baseline concentration, the magnitude of biological variation, and the magnitude of the required increment defined in the criterion. The lengths of the tumour detection times also depended on the rates of marker increase. **CONCLUSIONS:** The results suggest that different types of criteria should be used within different intervals of below cut-off level concentrations if the rate of false positive signals of marker increments should be kept $\leq 2\%$.

McArt, D. G., J. K. Blayney, et al. "PICan: An integromics framework for dynamic cancer biomarker discovery." *Mol Oncol*. 2015 Jun;9(6):1234-40. doi: 10.1016/j.molonc.2015.02.002. Epub 2015 Mar 4.

Modern cancer research on prognostic and predictive biomarkers demands the integration of established and emerging high-throughput technologies. However, these data are meaningless unless carefully integrated with patient clinical outcome and epidemiological information. Integrated datasets hold the key to discovering new biomarkers and therapeutic targets in cancer. We have developed a novel approach and set of methods for integrating and interrogating phenomic, genomic and clinical data sets to facilitate cancer biomarker discovery and patient stratification. Applied to a known paradigm, the biological and clinical relevance of TP53, PICan was able to recapitulate the known biomarker status and prognostic significance at a DNA, RNA and protein levels.

Mermelekas, G. and J. Zoidakis "Mass spectrometry-based membrane proteomics in cancer biomarker discovery." *Expert Rev Mol Diagn*. 2014 Jun;14(5):549-63. doi: 10.1586/14737159.2014.917965. Epub 2014 May 12.

Membrane proteins are involved in central processes such as cell signaling, cell-cell interactions and communication, ion and metabolite transport and in general play a crucial role in cell homeostasis. Cancer and cancer metastasis have been correlated to protein expression levels and dysfunction, with membrane proteins playing an important role, and are thus used as drug targets and potential biomarkers for prognostic or diagnostic purposes. Despite the critical biological significance of membrane proteins, proteomic analysis has been a challenging task due to their hydrophobicity. In this review, recent advances in the proteomic analysis of membrane proteins are presented, focusing on membrane fraction enrichment techniques combined with labeled or label-free shotgun proteomics approaches for the identification of potential cancer biomarkers.

Moein, M. M., D. Jabbar, et al. "A needle extraction utilizing a molecularly imprinted-sol-gel xerogel for on-line microextraction of the lung cancer biomarker bilirubin from plasma and urine samples." *J Chromatogr A*. 2014 Oct 31;1366:15-23. doi: 10.1016/j.chroma.2014.09.012. Epub 2014 Sep 26.

In the present work, a needle trap utilizing a molecularly imprinted sol-gel xerogel was prepared for the on-line microextraction of bilirubin from plasma and urine samples. Each prepared needle could be used for approximately one hundred extractions before it was discarded. Imprinted and non-imprinted sol-gel xerogel were applied for the extraction of bilirubin from plasma and urine samples. The produced molecularly imprinted sol-gel xerogel polymer showed high binding capacity and fast adsorption/desorption kinetics for bilirubin in plasma and urine samples. The adsorption capacity of molecularly imprinted sol-gel xerogel polymer was approximately 60% higher than that of non-imprinted polymer. The effect of the conditioning, washing and elution solvents, pH, extraction time, adsorption capacity and imprinting factor were investigated. The limit of detection and the lower limit of quantification were set to 1.6 and 5nmolL(-1), respectively using plasma or urine samples. The standard calibration curves were obtained within the concentration range of 5-1000nmolL(-1) in both plasma and urine samples. The coefficients of determination values (R(2)) were >=0.998 for all runs. The extraction recovery was approximately 80% for BR in the human plasma and urine samples.

Omenn, G. S., Y. Guan, et al. "A new class of protein cancer biomarker candidates: differentially expressed splice variants of ERBB2 (HER2/neu) and ERBB1 (EGFR) in breast cancer cell lines." *J Proteomics*. 2014 Jul 31;107:103-12. doi: 10.1016/j.jprot.2014.04.012. Epub 2014 May 5.

Combined RNA-Seq and proteomics analyses reveal striking differential expression of splice isoforms of key proteins in important cancer pathways and networks. Even between primary tumor cell lines from histologically similar inflammatory breast cancers, we find striking differences in hormone receptor-negative cell lines that are ERBB2 (Her2/neu)-amplified versus ERBB1 (EGFR) over-expressed with low ERBB2 activity. We have related these findings to protein-protein interaction networks, signaling and metabolic pathways, and methods for predicting functional variants among multiple alternative isoforms. Understanding the upstream ligands and regulators and the downstream pathways and interaction networks for ERBB receptors is certain to be important for explanation and prediction of the

variable levels of expression and therapeutic responses of ERBB+tumors in the breast and in other organ sites. Alternative splicing is a remarkable evolutionary development that increases protein diversity from multi-exonic genes without requiring expansion of the genome. It is no longer sufficient to report the up- or down-expression of genes and proteins without dissecting the complexity due to alternative splicing. This article is part of a Special Issue entitled: 20Years of Proteomics in memory of Viatliano Pallini. Guest Editors: Luca Bini, Juan J. Calvete, Natacha Turck, Denis Hochstrasser and Jean-Charles Sanchez.

Patel, S. and S. Ahmed "Emerging field of metabolomics: big promise for cancer biomarker identification and drug discovery." *J Pharm Biomed Anal*. 2015 Mar 25;107:63-74. doi: 10.1016/j.jpba.2014.12.020. Epub 2014 Dec 22.

Most cancers are lethal and metabolic alterations are considered a hallmark of this deadly disease. Genomics and proteomics have contributed vastly to understand cancer biology. Still there are missing links as downstream to them molecular divergence occurs. Metabolomics, the omic science that furnishes a dynamic portrait of metabolic profile is expected to bridge these gaps and boost cancer research. Metabolites being the end products are more stable than mRNAs or proteins. Previous studies have shown the efficacy of metabolomics in identifying biomarkers associated with diagnosis, prognosis and treatment of cancer. Metabolites are highly informative about the functional status of the biological system, owing to their proximity to organismal phenotypes. Scores of publications have reported about high-throughput data generation by cutting-edge analytic platforms (mass spectrometry and nuclear magnetic resonance). Further sophisticated statistical softwares (chemometrics) have enabled meaningful information extraction from the metabolomic data. Metabolomics studies have demonstrated the perturbation in glycolysis, tricarboxylic acid cycle, choline and fatty acid metabolism as traits of cancer cells. This review discusses the latest progress in this field, the future trends and the deficiencies to be surmounted for optimally implementation in oncology. The authors scoured through the most recent, high-impact papers archived in Pubmed, ScienceDirect, Wiley and Springer databases to compile this review to pique the interest of researchers towards cancer metabolomics.

Patris, S., P. De Pauw, et al. "Nanoimmunoassay onto a screen printed electrode for HER2 breast cancer biomarker determination." *Talanta*. 2014 Dec;130:164-70. doi: 10.1016/j.talanta.2014.06.069. Epub 2014 Jul 7.

A chip format sandwich-type immunoassay based on Nanobodies((R)) (Nbs) with the Human Epidermal Growth Factor Receptor (HER2) extracellular domain as antigen model has been developed. The HER2 is considered as an important biomarker because its overexpression causes an aggressive type of breast cancer. Nbs are single domain antigen-binding fragments derived from camelid heavy-chain antibodies. The strategy of the presently developed sandwich immunoassay takes advantage of the small size of Nbs for the detection of the electroactive redox tracer onto the screen printed electrode (SPE). A capture anti HER2 Nb was covalently immobilized onto the SPE, and the detection Nb, raised against another epitope of HER2, was labeled with horseradish peroxidase (HRP). The biosensor signal corresponded to the electroreduction of para-quinone generated at the SPE by the HRP in the presence of hydroquinone and hydrogen peroxide. The best performing and optimized immunoassay conditions consisted of 2 and 20 min for the first and the second incubation times, respectively. The amperometric signal obtained was proportional to the logarithm of HER2 concentration between 1 and 200 microg/mL and the modified SPE storage stability lasted for at least three weeks. Determination of HER2 in human cells has been realized.

Prieto, D. A., D. J. Johann, Jr., et al. "Mass spectrometry in cancer biomarker research: a case for immunodepletion of abundant blood-derived proteins from clinical tissue specimens." *Biomark Med.* 2014;8(2):269-86. doi: [10.2217/bmm.13.101](https://doi.org/10.2217/bmm.13.101).

The discovery of clinically relevant cancer biomarkers using mass spectrometry (MS)-based proteomics has proven difficult, primarily because of the enormous dynamic range of blood-derived protein concentrations and the fact that the 22 most abundant blood-derived proteins constitute approximately 99% of the total plasma protein mass. Immunodepletion of clinical body fluid specimens (e.g., serum/plasma) for the removal of highly abundant proteins is a reasonable and reproducible solution. Often overlooked, clinical tissue specimens also contain a formidable amount of highly abundant blood-derived proteins present in tissue-embedded networks of blood/lymph capillaries and interstitial fluid. Hence, the dynamic range impediment to biomarker discovery remains a formidable obstacle, regardless of clinical sample type (solid tissue and/or body fluid). Thus, we optimized and applied simultaneous immunodepletion of blood-derived proteins from solid tissue and peripheral blood, using clear cell renal cell carcinoma as a model disease. Integrative analysis of data from this approach and genomic data obtained from the same type of tumor revealed concordant key pathways

and protein targets germane to clear cell renal cell carcinoma. This includes the activation of the lipogenic pathway characterized by increased expression of adipophilin (PLIN2) along with 'cadherin switching', a phenomenon indicative of transcriptional reprogramming linked to renal epithelial dedifferentiation. We also applied immunodepletion of abundant blood-derived proteins to various tissue types (e.g., adipose tissue and breast tissue) showing unambiguously that the removal of abundant blood-derived proteins represents a powerful tool for the reproducible profiling of tissue proteomes. Herein, we show that the removal of abundant blood-derived proteins from solid tissue specimens is of equal importance to depletion of body fluids and recommend its routine use in the context of biological discovery and/or cancer biomarker research. Finally, this perspective presents the background, rationale and strategy for using tissue-directed high-resolution/accuracy MS-based shotgun proteomics to detect genuine tumor proteins in the peripheral blood of a patient diagnosed with nonmetastatic cancer, employing concurrent liquid chromatography-MS analysis of immunodepleted clinical tissue and blood specimens.

Reumer, A., E. Maes, et al. "Colorectal cancer biomarker discovery and validation using LC-MS/MS-based proteomics in blood: truth or dare?" *Expert Rev Proteomics.* 2014 Aug;11(4):449-63. doi: [10.1586/14789450.2014.905743](https://doi.org/10.1586/14789450.2014.905743). Epub 2014 Apr 7.

Globally, colorectal cancer (CRC) is the third most common malignant neoplasm. However, highly sensitive, specific, noninvasive tests that allow CRC diagnosis at an early stage are still needed. As circulatory blood reflects the physiological status of an individual and/or the disease status for several disorders, efforts have been undertaken to identify candidate diagnostic CRC markers in plasma and serum. In this review, the challenges, bottlenecks and promising properties of mass spectrometry (MS)-based proteomics in blood are discussed. More specifically, important aspects in clinical design, sample retrieval, sample preparation, and MS analysis are presented. The recent developments in targeted MS approaches in plasma or serum are highlighted as well.

Santos, G. M., F. Zhao, et al. "Label-free, zeptomole cancer biomarker detection by surface-enhanced fluorescence on nanoporous gold disk plasmonic nanoparticles." *J Biophotonics.* 2015 Feb 26;9999(9999). doi: [10.1002/jbio.201400134](https://doi.org/10.1002/jbio.201400134).

We experimentally demonstrate a label-free biosensor for the ERBB2 cancer gene DNA target based on the distance-dependent detection of surface-enhanced fluorescence (SEF) on nanoporous gold disk

(NPGD) plasmonic nanoparticles. We achieve detection of 2.4 zeptomole of DNA target on the NPGD substrate with an upper concentration detection limit of 1 nM. Without the use of molecular spacers, the NPGD substrate as an SEF platform was shown to provide higher net fluorescence for visible and NIR fluorophores compared to glass and non-porous gold substrates. The enhanced fluorescence signals in patterned nanoporous gold nanoparticles make NPGD a viable material for further reducing detection limits for biomolecular targets used in clinical assays. With patterned nanoporous gold disk (NPGD) plasmonic nanoparticles, a label-free biosensor that makes use of distance-dependent detection of surface-enhanced fluorescence (SEF) is constructed and tested for zeptomole detection of ERBB2 cancer gene DNA targets.

Sharma, A., S. Hong, et al. "Single-walled carbon nanotube based transparent immunosensor for detection of a prostate cancer biomarker osteopontin." Anal Chim Acta. 2015 Apr 15;869:68-73. doi: 10.1016/j.aca.2015.02.010. Epub 2015 Feb 11.

Osteopontin (OPN) is involved in almost all steps of cancer development, and it is being investigated as a potential biomarker for a diagnosis and prognosis of prostate cancer. Here, we report a label-free, highly sensitive and transparent immunosensor based on single-walled carbon nanotubes (SWCNTs) for detection of OPN. A high density of COOH functionalized SWCNTs was deposited between two gold/indium tin oxide electrodes on a glass substrate by dielectrophoresis. Monoclonal antibodies specific to OPN were covalently immobilized on the SWCNTs. Relative resistance change of the immunosensors was measured as the concentration of OPN in phosphate buffer saline (PBS) and human serum was varied from 1 pg mL⁻¹ to 1 μg mL⁻¹ for different channel lengths of 2, 5, and 10 μm, showing a highly linear and reproducible behavior (R²>97%). These immunosensors were also specific to OPN against another test protein, bovine serum albumin, PBS and human serum, showing that a limit of detection for OPN was 0.3 pg mL⁻¹. This highly sensitive and transparent immunosensor has a great potential as a simple point-of-care test kit for various protein biomarkers.

Shetty, R. K., S. K. Bhandary, et al. "Significance of Serum L-fucose Glycoprotein as Cancer Biomarker in Head and Neck Malignancies without Distant Metastasis." J Clin Diagn Res. 2013 Dec;7(12):2818-20. doi: 10.7860/JCDR/2013/6681.3765. Epub 2013 Dec 15.

BACKGROUND: Head and neck neoplasia is a major form of cancer in India, accounting for 30% of

all cancers which occur in males and 11% of cancers which occur in females. Elevated serum L-fucose glycoprotein levels have been reported to be associated with neoplastic conditions involving various sites. Therefore, monitoring serum/tissue L-fucose glycoprotein levels could be a promising approach for the early diagnosis and prognosis of head neck cancers. AIM: This study was carried out to determine the significance of serum L-fucose levels in head and neck malignancies. SETTINGS AND DESIGN: This comparative study was carried out at a tertiary care hospital in South India. MATERIAL AND METHODS: Serum L-fucose glycoprotein levels were estimated in 50 patients with histopathologically confirmed head and neck malignancies and they were compared with those of 50 age- and sex-matched healthy controls. STATISTICAL ANALYSIS USED: Student's t-test was used to compare L-fucose glycoprotein levels in study and control groups. RESULTS: Most common site of the primary tumour in the current study was oral cavity, followed by larynx, hypopharynx and oropharynx respectively. Comparison of glycoprotein L-fucose in two groups showed more than a two-fold rise in serum fucose levels in cases as compared to the those in controls, with mean values of 11.33±7.39 and 4.74±1.55 mg% in cases and controls respectively. There was no relationship between serum fucose levels and age, sex and tumour differentiation. CONCLUSION: Serum glycoprotein L-fucose levels can be used as an effective biochemical indicator in conjunction with clinical diagnostic procedures in head and neck neoplasia and they may be useful for monitoring recurrences.

Silsirivanit, A., K. Sawanyawisuth, et al. "Cancer biomarker discovery for cholangiocarcinoma: the high-throughput approaches." J Hepatobiliary Pancreat Sci. 2014 Jun;21(6):388-96. doi: 10.1002/jhbp.68. Epub 2014 Feb 12.

Cholangiocarcinoma (CCA) is difficult to diagnose at an early stage and most tumors are detected at late stage where surgery or other therapy is ineffective. Many advanced techniques are applied to diagnose CCA; however, most are expensive and have varying degrees of accuracy. A less invasive and simpler procedure such as serum markers would be of substantial clinical benefit for diagnosis, monitoring, and predicting outcome for CCA patients. Recent advances in "Omics" technologies offer remarkable opportunities for establishment of biomarker-related to diseases. In this review, the potential biomarkers obtained from proteomics and glycomic studies are evaluated. Several protein markers were discovered from patient specimen, using two dimensional-differential gel electrophoresis couple with liquid

chromatography tandem mass spectrometry (2D-DIGE/LC-MS-MS), matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS), surface enhanced laser desorption/ionization (SELDI)-TOF-MS and capillary electrophoresis (CE)-MS, etc. Newly reported CCA-associated glyco-biomarkers were identified using lectin-assisted, monoclonal antibody-assisted or specific-target strategies. The combination between carbohydrate binding-lectin and core protein-binding mAb significantly increased the values for detection of the glyco-biomarkers for CCA. Searching for specific and sensitive molecular markers to be used for population screening is worth being evaluated. This could lead to earlier diagnosis and improve outcome. Further investigation of those biomarker functions is also of value in order to better understand the tumor biology and use them as targets for future therapeutic agents.

Sivakumar, S. and S. Niranjali Devaraj "Tertiary structure prediction and identification of druggable pocket in the cancer biomarker - Osteopontin-c." J Diabetes Metab Disord. 2014 Jan 8;13(1):13. doi: [10.1186/2251-6581-13-13](https://doi.org/10.1186/2251-6581-13-13).

BACKGROUND: Osteopontin (Eta, secreted sialoprotein 1, opn) is secreted from different cell types including cancer cells. Three splice variant forms namely osteopontin-a, osteopontin-b and osteopontin-c have been identified. The main astonishing feature is that osteopontin-c is found to be elevated in almost all types of cancer cells. This was the vital point to consider it for sequence analysis and structure predictions which provide ample chances for prognostic, therapeutic and preventive cancer research. **METHODS:** Osteopontin-c gene sequence was determined from Breast Cancer sample and was translated to protein sequence. It was then analyzed using various software and web tools for binding pockets, docking and druggability analysis. Due to the lack of homological templates, tertiary structure was predicted using ab-initio method server - I-TASSER and was evaluated after refinement using web tools. Refined structure was compared with known bone sialoprotein electron microscopic structure and docked with CD44 for binding analysis and binding pockets were identified for drug designing. **RESULTS:** Signal sequence of about sixteen amino acid residues was identified using signal sequence prediction servers. Due to the absence of known structures of similar proteins, three dimensional structure of osteopontin-c was predicted using I-TASSER server. The predicted structure was refined with the help of SUMMA server and was validated using SAVES server. Molecular dynamic analysis was carried out using GROMACS software. The final model was built and was used for

docking with CD44. Druggable pockets were identified using pocket energies. **CONCLUSIONS:** The tertiary structure of osteopontin-c was predicted successfully using the ab-initio method and the predictions showed that osteopontin-c is of fibrous nature comparable to fibronectin. Docking studies showed the significant similarities of QSAET motif in the interaction of CD44 and osteopontins between the normal and splice variant forms of osteopontins and binding pockets analyses revealed several pockets which paved the way to the identification of a druggable pocket.

Sjostrom, M., R. Ossola, et al. "A Combined Shotgun and Targeted Mass Spectrometry Strategy for Breast Cancer Biomarker Discovery." J Proteome Res. 2015 Jun 5.

It is of highest importance to find proteins responsible for breast cancer dissemination, for use as biomarkers or treatment targets. We established and performed a combined nontargeted LC-MS/MS and a targeted LC-SRM workflow for discovery and validation of protein biomarkers. Eighty breast tumors, stratified for estrogen receptor status and development of distant recurrence (DR +/-), were collected. After enrichment of N-glycosylated peptides, label-free LC-MS/MS was performed on each individual tumor in triplicate. In total, 1515 glycopeptides from 778 proteins were identified and used to create a map of the breast cancer N-glycosylated proteome. Based on this specific proteome map, we constructed a 92-plex targeted label-free LC-SRM panel. These proteins were quantified across samples by LC-SRM, resulting in 10 proteins consistently differentially regulated between DR+/DR- tumors. Five proteins were further validated in a separate cohort as prognostic biomarkers at the gene expression level. We also compared the LC-SRM results to clinically reported HER2 status, demonstrating its clinical accuracy. In conclusion, we demonstrate a combined mass spectrometry strategy, at large scale on clinical samples, leading to the identification and validation of five proteins as potential biomarkers for breast cancer recurrence. All MS data are available via ProteomeXchange and PASSEL with identifiers PXD001685 and PASS00643.

Stephan, C., J. Wilkosz, et al. "Urinary thiosulfate as failed prostate cancer biomarker - an exemplary multicenter re-evaluation study." Clin Chem Lab Med. 2015 Feb;53(3):477-83. doi: [10.1515/cclm-2014-0729](https://doi.org/10.1515/cclm-2014-0729).

BACKGROUND: In 2013, thiosulfate in urine has been proposed as promising prostate cancer (PCa) biomarker. However, a missing comparison with other proven PCa markers suggested a re-evaluation study. Therefore, together with the authors

from the initial study, the diagnostic accuracy of thiosulfate was compared with that of urinary prostate cancer associated 3 (PCA3), serum prostate health index (Phi), and percent free prostate-specific antigen (%fPSA). Thiosulfate was further measured in a multicenter approach to exclude center-related biases. METHODS: Thiosulfate, calculated as ratio of thiosulfate to urinary creatinine (TS/Crea ratio), was measured in two cohorts in a total of 269 patients. In the retrospective study (n=160) PCA3, Phi, PSA, and %fPSA were compared with the TS/Crea ratio between patients with and without PCa according to the prostate needle biopsy results. The second prospective cohort included 109 patients from four centers. RESULTS: The median TS/Crea ratio was not statistically different between the patients with and without PCa. The receiver-operating characteristics showed that the TS/Crea ratio was unable to discriminate between patients with and without PCa in contrast to %fPSA, Phi, and PCA3. In all four centers, the low median TS/Crea ratios (<1 mmol/mol) in both patient cohorts were confirmed and thiosulfate was again not able to distinguish between them (p-values, 0.13-0.90). CONCLUSIONS: This study could not confirm the previously observed high median TS/Crea ratio in PCa patients in comparison to non-PCa patients. Thiosulfate subsequently failed as PCa biomarker while PCA3 and Phi showed the expected diagnostic improvement.

Taniguchi, N. and Y. Kizuka "Glycans and cancer: role of N-glycans in cancer biomarker, progression and metastasis, and therapeutics." Adv Cancer Res. 2015;126:11-51. doi: 10.1016/bs.acr.2014.11.001. Epub 2015 Feb 7.

Glycosylation is catalyzed by various glycosyltransferase enzymes which are mostly located in the Golgi apparatus in cells. These enzymes glycosylate various complex carbohydrates such as glycoproteins, glycolipids, and proteoglycans. The enzyme activity of glycosyltransferases and their gene expression are altered in various pathophysiological situations including cancer. Furthermore, the activity of glycosyltransferases is controlled by various factors such as the levels of nucleotide sugars, acceptor substrates, nucleotide sugar transporters, chaperons, and endogenous lectin in cancer cells. The glycosylation results in various functional changes of glycoproteins including cell surface receptors and adhesion molecules such as E-cadherin and integrins. These changes confer the unique characteristic phenotypes associated with cancer cells. Therefore, glycans play key roles in cancer progression and treatment. This review focuses on glycan structures, their biosynthetic glycosyltransferases, and their genes in relation to their biological significance and

involvement in cancer, especially cancer biomarkers, epithelial-mesenchymal transition, cancer progression and metastasis, and therapeutics. Major N-glycan branching structures which are directly related to cancer are beta1,6-GlcNAc branching, bisecting GlcNAc, and core fucose. These structures are enzymatic products of glycosyltransferases, GnT-V, GnT-III, and Fut8, respectively. The genes encoding these enzymes are designated as MGAT5 (Mgat5), MGAT3 (Mgat3), and FUT8 (Fut8) in humans (mice in parenthesis), respectively. GnT-V is highly associated with cancer metastasis, whereas GnT-III is associated with cancer suppression. Fut8 is involved in expression of cancer biomarker as well as in the treatment of cancer. In addition to these enzymes, GnT-IV and GnT-IX (GnT-Vb) will be also discussed in relation to cancer.

Tavallaie, R., S. R. De Almeida, et al. "Toward biosensors for the detection of circulating microRNA as a cancer biomarker: an overview of the challenges and successes." Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2015 Jul;7(4):580-92. doi: 10.1002/wnan.1324. Epub 2014 Dec 22.

Considerable attention has been dedicated to developing feasible point-of-care tests for cancer diagnosis and prognosis. An ideal biomarker for clinical use should be easily assayed with minimally invasive medical procedures but possess high sensitivity and specificity. The role of microRNAs (miRNAs) in the regulation of different cellular processes, the unique altered patterns in cancer patients and presence in body fluids in the stable form, points to their clinical utility as blood-based biomarkers for diagnosis, prognosis, and treatment of cancer. Although a variety of selective and sensitive laboratory-based methods are already exist for the detection of circulating miRNA, having a simple, low-cost and rapid assay, which could be routinely used in clinical practice, is still required. Among different approaches that have developed for circulating miRNA detection, biosensors, due to the high sensitivity, ease of use, short assay time, non-toxic experimental steps, and adaptability to point-of-care testing, exhibit very attractive properties for developing portable devices. With this view, we present an overview of some of the challenges that still need to be met to be able to use circulating miRNAs in clinical practice, including their clinical significance, sample preparation, and detection. In particular, we highlight the recent advances in the rapidly developing area of biosensors for circulating miRNA detection, along with future prospects and challenges. WIREs Nanomed Nanobiotechnol 2015, 7:580-592. doi: 10.1002/wnan.1324 For further resources related to this article, please visit the WIREs website.

Theiss, A. P., D. Chafin, et al. "Immunohistochemistry of colorectal cancer biomarker phosphorylation requires controlled tissue fixation." *PLoS One*. 2014 Nov 19;9(11):e113608. doi: 10.1371/journal.pone.0113608. eCollection 2014.

Phosphorylated signaling molecules are biomarkers of cancer pathophysiology and resistance to therapy, but because phosphoprotein analytes are often labile, poorly controlled clinical laboratory practices could prevent translation of research findings in this area from the bench to the bedside. We therefore compared multiple biomarker and phosphoprotein immunohistochemistry (IHC) results in 23 clinical colorectal carcinoma samples after either a novel, rapid tissue fixation protocol or a standard tissue fixation protocol employed by clinical laboratories, and we also investigated the effect of a defined post-operative "cold" ischemia period on these IHC results. We found that a one-hour cold ischemia interval, allowed by ASCO/CAP guidelines for certain cancer biomarker assays, is highly deleterious to certain phosphoprotein analytes, specifically the phosphorylated epidermal growth factor receptor (pEGFR), but shorter ischemic intervals (less than 17 minutes) facilitate preservation of phosphoproteins. Second, we found that a rapid 4-hour, two temperature, formalin fixation yielded superior staining in several cases with select markers (pEGFR, pBAD, pAKT) compared to a standard overnight room temperature fixation protocol, despite taking less time. These findings indicate that the future research and clinical utilities of phosphoprotein IHC for assessing colorectal carcinoma pathophysiology absolutely depend upon attention to preanalytical factors and rigorously controlled tissue fixation protocols.

Truta, L. A. and M. G. Sales "Sol-gel chemistry in biosensing devices of electrical transduction: application to CEA cancer biomarker." *Curr Top Med Chem*. 2015;15(3):256-61.

Sol-gel chemistry allows the immobilization of organic molecules of biological origin on suitable solid supports, permitting their integration into biosensing devices widening the possibility of local applications. The present work is an application of this principle, where the link between electrical receptor platform and the antibody acting as biorecognition element is made by sol-gel chemistry. The immunosensor design was targeted for carcinoembryonic antigen (CEA), an important biomarker for screening the colorectal cancer, by electrochemical techniques, namely electrochemical impedance spectroscopy (EIS) and square wave voltammetry (SVW). The device displayed linear behavior to CEA in EIS and in SWV assays ranging

from 0.50 to 1.5ng/mL, and 0.25 to 1.5ng/mL, respectively. The corresponding detection limits were 0.42 and 0.043 ng/mL. Raman spectroscopy was used to characterize the surface modifications on the conductive platform (FTO glass). Overall, simple sol-gel chemistry was effective at the biosensing design and the presented approach can be a potential method for screening CEA in point-of-care, due to the simplicity of fabrication, short response time and low cost.

Wittmann, B. M., S. M. Stirdivant, et al. "Bladder cancer biomarker discovery using global metabolomic profiling of urine." *PLoS One*. 2014 Dec 26;9(12):e115870. doi: 10.1371/journal.pone.0115870. eCollection 2014.

Bladder cancer (BCa) is a common malignancy worldwide and has a high probability of recurrence after initial diagnosis and treatment. As a result, recurrent surveillance, primarily involving repeated cystoscopies, is a critical component of post diagnosis patient management. Since cystoscopy is invasive, expensive and a possible deterrent to patient compliance with regular follow-up screening, new non-invasive technologies to aid in the detection of recurrent and/or primary bladder cancer are strongly needed. In this study, mass spectrometry based metabolomics was employed to identify biochemical signatures in human urine that differentiate bladder cancer from non-cancer controls. Over 1000 distinct compounds were measured including 587 named compounds of known chemical identity. Initial biomarker identification was conducted using a 332 subject sample set of retrospective urine samples (cohort 1), which included 66 BCa positive samples. A set of 25 candidate biomarkers was selected based on statistical significance, fold difference and metabolic pathway coverage. The 25 candidate biomarkers were tested against an independent urine sample set (cohort 2) using random forest analysis, with palmitoyl sphingomyelin, lactate, adenosine and succinate providing the strongest predictive power for differentiating cohort 2 cancer from non-cancer urines. Cohort 2 metabolite profiling revealed additional metabolites, including arachidonate, that were higher in cohort 2 cancer vs. non-cancer controls, but were below quantitation limits in the cohort 1 profiling. Metabolites related to lipid metabolism may be especially interesting biomarkers. The results suggest that urine metabolites may provide a much needed non-invasive adjunct diagnostic to cystoscopy for detection of bladder cancer and recurrent disease management.

The above contents are the collected information from Internet and public resources to offer to the

people for the convenient reading and information disseminating and sharing.

References

- Alisoltani, A., H. Fallahi, et al. "RNA-seq SSRs and small RNA-seq SSRs: new approaches in cancer biomarker discovery." *Gene*. 2015 Apr 10;560(1):34-43. doi: 10.1016/j.gene.2015.01.027. Epub 2015 Jan 30.
- Cao, G., G. Hajisalem, et al. "Quantification of an exogenous cancer biomarker in urinalysis by Raman spectroscopy." *Analyst*. 2014 Nov 7;139(21):5375-8. doi: 10.1039/c4an01309c.
- Chambers, A. G., A. J. Percy, et al. "MRM for the verification of cancer biomarker proteins: recent applications to human plasma and serum." *Expert Rev Proteomics*. 2014 Apr;11(2):137-48. doi: 10.1586/14789450.2014.877346. Epub 2014 Jan 29.
- Delgado, A. P., S. Hamid, et al. "A novel transmembrane glycoprotein cancer biomarker present in the X chromosome." *Cancer Genomics Proteomics*. 2014 Mar-Apr;11(2):81-92.
- Derks, S., A. H. Cleven, et al. "Emerging evidence for CHFR as a cancer biomarker: from tumor biology to precision medicine." *Cancer Metastasis Rev*. 2014 Mar;33(1):161-71. doi: 10.1007/s10555-013-9462-4.
- Devi, R. V., M. Doble, et al. "Nanomaterials for early detection of cancer biomarker with special emphasis on gold nanoparticles in immunoassays/sensors." *Biosens Bioelectron*. 2015 Jun 15;68:688-98. doi: 10.1016/j.bios.2015.01.066. Epub 2015 Jan 29.
- Eaton, R. M., J. A. Shallcross, et al. "Selection of DNA aptamers for ovarian cancer biomarker HE4 using CE-SELEX and high-throughput sequencing." *Anal Bioanal Chem*. 2015 Apr 12.
- Egleton, J. E., C. C. Thinnies, et al. "Structure-activity relationships and colorimetric properties of specific probes for the putative cancer biomarker human arylamine N-acetyltransferase 1." *Bioorg Med Chem*. 2014 Jun 1;22(11):3030-54. doi: 10.1016/j.bmc.2014.03.015. Epub 2014 Mar 28.
- Eletxigerra, U., J. Martinez-Perdiguerro, et al. "Amperometric magnetoimmunosensor for ErbB2 breast cancer biomarker determination in human serum, cell lysates and intact breast cancer cells." *Biosens Bioelectron*. 2015 Aug 15;70:34-41. doi: 10.1016/j.bios.2015.03.017. Epub 2015 Mar 10.
- Emami, M., M. Shamsipur, et al. "An electrochemical immunosensor for detection of a breast cancer biomarker based on antiHER2-iron oxide nanoparticle bioconjugates." *Analyst*. 2014 Jun 7;139(11):2858-66. doi: 10.1039/c4an00183d.
- Espinoza-Castaneda, M., A. de la Escosura-Muniz, et al. "Nanochannel array device operating through Prussian blue nanoparticles for sensitive label-free immunodetection of a cancer biomarker." *Biosens Bioelectron*. 2015 May 15;67:107-14. doi: 10.1016/j.bios.2014.07.039. Epub 2014 Jul 24.
- Gogalic, S., U. Sauer, et al. "Bladder cancer biomarker array to detect aberrant levels of proteins in urine." *Analyst*. 2015 Feb 7;140(3):724-35. doi: 10.1039/c4an01432d.
- Hadad, S. M., P. Coates, et al. "Evidence for biological effects of metformin in operable breast cancer: biomarker analysis in a pre-operative window of opportunity randomized trial." *Breast Cancer Res Treat*. 2015 Feb;150(1):149-55. doi: 10.1007/s10549-015-3307-5. Epub 2015 Feb 15.
- Haldrup, C., N. Kosaka, et al. "Profiling of circulating microRNAs for prostate cancer biomarker discovery." *Drug Deliv Transl Res*. 2014 Feb;4(1):19-30. doi: 10.1007/s13346-013-0169-4.
- Hamelin-Peyron, C., V. Vlaeminck-Guillem, et al. "Prostate cancer biomarker annexin A3 detected in urines obtained following digital rectal examination presents antigenic variability." *Clin Biochem*. 2014 Jul;47(10-11):901-8. doi: 10.1016/j.clinbiochem.2014.05.063. Epub 2014 Jun 2.
- Henrique, R. and C. Jeronimo "DNA hypomethylation in plasma as a cancer biomarker: when less is more?" *Expert Rev Mol Diagn*. 2014 May;14(4):419-22. doi: 10.1586/14737159.2014.905203. Epub 2014 Apr 1.
- Kadimisetty, K., S. Malla, et al. "Automated multiplexed ECL Immunoarrays for cancer biomarker proteins." *Anal Chem*. 2015 Apr 21;87(8):4472-8. doi: 10.1021/acs.analchem.5b00421. Epub 2015 Apr 9.
- Kamal, A. H., S. Power, et al. "Optimizing the quality of breast cancer biomarker use at Duke Cancer Institute." *J Natl Compr Canc Netw*. 2014 Feb;12 Suppl 1:S21-4.
- Kumar, S., A. Nag, et al. "A Comprehensive Review on miR-200c, a Promising Cancer Biomarker with Therapeutic Potential." *Curr Drug Targets*. 2015 Mar 25.
- Lastraioli, E., J. Iorio, et al. Ion channel expression as promising cancer biomarker, *Biochim Biophys Acta*. 2014 Dec 24. pii: S0005-2736(14)00452-0. doi: 10.1016/j.bbamem.2014.12.016.
- Lund, F., P. H. Petersen, et al. "Criteria to interpret cancer biomarker increments crossing the recommended cut-off compared in a simulation model focusing on false positive signals and tumour detection time." *Clin Chim Acta*. 2014 Apr 20;431:192-7. doi: 10.1016/j.cca.2014.01.013. Epub 2014 Feb 6.
- Ma H, Chen G. Stem cell. *The Journal of American Science* 2005;1(2):90-92.
- Ma H, Cherg S. Eternal Life and Stem Cell. *Nature and Science*. 2007;5(1):81-96.
- Ma H, Cherg S. Nature of Life. *Life Science Journal* 2005;2(1):7 - 15.
- Ma H, Yang Y. Turratopsis nutricula. *Nature and Science* 2010;8(2):15-20. http://www.sciencepub.net/nature/ns0802/03_1279_ho_ngbao_turratopsis_ns0802_15_20.pdf.
- Ma H. The Nature of Time and Space. *Nature and science* 2003;1(1):1-11. *Nature and science* 2007;5(1):81-96.
- McArt, D. G., J. K. Blayney, et al. "PICan: An integromics framework for dynamic cancer biomarker discovery." *Mol Oncol*. 2015 Jun;9(6):1234-40. doi: 10.1016/j.molonc.2015.02.002. Epub 2015 Mar 4.
- Mermelekas, G. and J. Zoidakis "Mass spectrometry-based membrane proteomics in cancer biomarker discovery." *Expert Rev Mol Diagn*. 2014

- Jun;14(5):549-63. doi: [10.1586/14737159.2014.917965](https://doi.org/10.1586/14737159.2014.917965). Epub 2014 May 12.
29. Moein, M. M., D. Jabbar, et al. "A needle extraction utilizing a molecularly imprinted-sol-gel xerogel for on-line microextraction of the lung cancer biomarker bilirubin from plasma and urine samples." *J Chromatogr A*. 2014 Oct 31;1366:15-23. doi: [10.1016/j.chroma.2014.09.012](https://doi.org/10.1016/j.chroma.2014.09.012). Epub 2014 Sep 26.
 30. National Center for Biotechnology Information, U.S. National Library of Medicine. <http://www.ncbi.nlm.nih.gov/pubmed>. 2015.
 31. Omenn, G. S., Y. Guan, et al. "A new class of protein cancer biomarker candidates: differentially expressed splice variants of ERBB2 (HER2/neu) and ERBB1 (EGFR) in breast cancer cell lines." *J Proteomics*. 2014 Jul 31;107:103-12. doi: [10.1016/j.jprot.2014.04.012](https://doi.org/10.1016/j.jprot.2014.04.012). Epub 2014 May 5.
 32. Patel, S. and S. Ahmed "Emerging field of metabolomics: big promise for cancer biomarker identification and drug discovery." *J Pharm Biomed Anal*. 2015 Mar 25;107:63-74. doi: [10.1016/j.jpba.2014.12.020](https://doi.org/10.1016/j.jpba.2014.12.020). Epub 2014 Dec 22.
 33. Patris, S., P. De Pauw, et al. "Nanoimmunoassay onto a screen printed electrode for HER2 breast cancer biomarker determination." *Talanta*. 2014 Dec;130:164-70. doi: [10.1016/j.talanta.2014.06.069](https://doi.org/10.1016/j.talanta.2014.06.069). Epub 2014 Jul 7.
 34. Prieto, D. A., D. J. Johann, Jr., et al. "Mass spectrometry in cancer biomarker research: a case for immunodepletion of abundant blood-derived proteins from clinical tissue specimens." *Biomark Med*. 2014;8(2):269-86. doi: [10.2217/bmm.13.101](https://doi.org/10.2217/bmm.13.101).
 35. Reumer, A., E. Maes, et al. "Colorectal cancer biomarker discovery and validation using LC-MS/MS-based proteomics in blood: truth or dare?" *Expert Rev Proteomics*. 2014 Aug;11(4):449-63. doi: [10.1586/14789450.2014.905743](https://doi.org/10.1586/14789450.2014.905743). Epub 2014 Apr 7.
 36. Santos, G. M., F. Zhao, et al. "Label-free, zeptomole cancer biomarker detection by surface-enhanced fluorescence on nanoporous gold disk plasmonic nanoparticles." *J Biophotonics*. 2015 Feb 26;9999(9999). doi: [10.1002/jbio.201400134](https://doi.org/10.1002/jbio.201400134).
 37. Sharma, A., S. Hong, et al. "Single-walled carbon nanotube based transparent immunosensor for detection of a prostate cancer biomarker osteopontin." *Anal Chim Acta*. 2015 Apr 15;869:68-73. doi: [10.1016/j.aca.2015.02.010](https://doi.org/10.1016/j.aca.2015.02.010). Epub 2015 Feb 11.
 38. Shetty, R. K., S. K. Bhandary, et al. "Significance of Serum L-fucose Glycoprotein as Cancer Biomarker in Head and Neck Malignancies without Distant Metastasis." *J Clin Diagn Res*. 2013 Dec;7(12):2818-20. doi: [10.7860/JCDR/2013/6681.3765](https://doi.org/10.7860/JCDR/2013/6681.3765). Epub 2013 Dec 15.
 39. Silsirivanit, A., K. Sawanyawisuth, et al. "Cancer biomarker discovery for cholangiocarcinoma: the high-throughput approaches." *J Hepatobiliary Pancreat Sci*. 2014 Jun;21(6):388-96. doi: [10.1002/jhbp.68](https://doi.org/10.1002/jhbp.68). Epub 2014 Feb 12.
 40. Sivakumar, S. and S. Niranjali Devaraj "Tertiary structure prediction and identification of druggable pocket in the cancer biomarker - Osteopontin-c." *J Diabetes Metab Disord*. 2014 Jan 8;13(1):13. doi: [10.1186/2251-6581-13-13](https://doi.org/10.1186/2251-6581-13-13).
 41. Sjoström, M., R. Ossola, et al. "A Combined Shotgun and Targeted Mass Spectrometry Strategy for Breast Cancer Biomarker Discovery." *J Proteome Res*. 2015 Jun 5.
 42. Stephan, C., J. Wilkosz, et al. "Urinary thiosulfate as failed prostate cancer biomarker - an exemplary multicenter re-evaluation study." *Clin Chem Lab Med*. 2015 Feb;53(3):477-83. doi: [10.1515/cclm-2014-0729](https://doi.org/10.1515/cclm-2014-0729).
 43. Taniguchi, N. and Y. Kizuka "Glycans and cancer: role of N-glycans in cancer biomarker, progression and metastasis, and therapeutics." *Adv Cancer Res*. 2015;126:11-51. doi: [10.1016/bs.acr.2014.11.001](https://doi.org/10.1016/bs.acr.2014.11.001). Epub 2015 Feb 7.
 44. Tavalalaie, R., S. R. De Almeida, et al. "Toward biosensors for the detection of circulating microRNA as a cancer biomarker: an overview of the challenges and successes." *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2015 Jul;7(4):580-92. doi: [10.1002/wnan.1324](https://doi.org/10.1002/wnan.1324). Epub 2014 Dec 22.
 45. Theiss, A. P., D. Chafin, et al. "Immunohistochemistry of colorectal cancer biomarker phosphorylation requires controlled tissue fixation." *PLoS One*. 2014 Nov 19;9(11):e113608. doi: [10.1371/journal.pone.0113608](https://doi.org/10.1371/journal.pone.0113608). eCollection 2014.
 46. Truta, L. A. and M. G. Sales "Sol-gel chemistry in biosensing devices of electrical transduction: application to CEA cancer biomarker." *Curr Top Med Chem*. 2015;15(3):256-61.
 47. Wikipedia. The free encyclopedia. <http://en.wikipedia.org>. 2015.
 48. Wittmann, B. M., S. M. Stirdivant, et al. "Bladder cancer biomarker discovery using global metabolomic profiling of urine." *PLoS One*. 2014 Dec 26;9(12):e115870. doi: [10.1371/journal.pone.0115870](https://doi.org/10.1371/journal.pone.0115870). eCollection 2014.

5/15/2015