TYMORA
Analytical Operations

TECHNOLOGY SUMMARY AND VALUE PROPOSITION

Market Opportunity

The use of blood, urine and other biofluids to diagnose, monitor and manage cancer is fundamental to medical diagnostics. According to BCC Research, the global liquid biopsy market is expected to reach $4.5B by 2022 and is primarily comprised of CTCs (circulating tumor cells) and ctDNA (circulating tumor DNA). A major limitation of these methods is the relatively low level of these analytes in blood, especially at an early stage of the disease. Current approaches also overwhelmingly focus on genetic information – usually gene mutations. Genome information can be prognostic but is often confounded by the layers of regulation that exist between DNA and an expressed phenotype. Protein analysis provides real-time information about the organism's physiological functions and disease progression. Compared to gene panel testing, immunoassays are also relatively inexpensive and are more likely to get reimbursed. Nonetheless, genetic screens dominate the market because the technology for genomic readout and reproducible quantitation is readily available and highly robust.

Our company has recently developed a similarly robust process for the identification and detection of new biomarkers based on proteins and protein phosphorylation – a true measure of dynamic activity and cellular signaling. Assay of phosphoproteins in tissue is challenging because protein phosphorylation is highly dynamic during the tissue biopsy process, and is undesirable because of the invasive nature of the procedure. In addition, a physiological tumor site must be already present for such an intervention, thus limiting diagnosis timeline or remission detection to a later stage than needed. Analysis of phosphoproteins in biofluids is even more challenging because of the presence of phosphatases. Given these obstacles and their typically low amounts, few phosphorylated proteins and other low-abundance proteins in raw plasma or urine can be readily detected.

By using cell-secreted extracellular vesicles (EVs), we have overcome these challenges. EVs originate from cellular membranes, are shed into every biological fluid, and provide a good representation of the protein content of the parent cells. The membrane surface of the EVs protects the proteins inside from external enzymes. EV-based disease markers can also be identified well before the onset of symptoms or physiological detection of a tumor, making them promising biomarker candidates for detecting early-stage cancer and other diseases.

Technology Summary

Given the immaturity of the EV analysis field, researchers have yet to standardize EV collection and processing. Most rely on differential centrifugation, with ultracentrifugation as the final step. However, this approach is very time consuming, costly, has low EV recovery, low-throughput and difficult to implement in a clinical setting.

We have developed a novel approach to analyze EV phosphoproteins and other low-abundant signaling molecules. The technology is based on non-antibody affinity beads and is termed EVtrap (Extracellular Vesicles total recovery and purification). By utilizing our optimized method, we are able to identify over a thousand of phosphoproteins from EVs from only 1mL of plasma or 10mL of urine, a significant improvement over anything previously possible (*see table on the right*). We have used this procedure to examine >150 urine and >100 plasma samples and produced hundreds of EV phosphoproteins in each case with obvious differences between cancer and healthy conditions.

	Best previous result	Our result
Plasma	58 phosphoproteins	>1,500 phosphoproteins
Urine	14 phosphoproteins	>1,100 phosphoproteins

Value Proposition

We have developed a reliable and effective platform for biofluid-based DNA/RNA, protein and phosphoprotein analysis using our EVtrap for EV isolation and cargo extraction approach. We propose to partner with other organizations for protein biomarker discovery and validation in a variety of cancers in different biofluids (e.g. for disease monitoring or companion diagnostics development). This approach would be particularly useful as a companion for kinase inhibitor-based therapies. Phosphorylation analysis in biofluid EVs can be used in two ways: a) to detect changes in cancer-induced phosphorylation itself in EV proteins; or b) to use phosphorylation as a surrogate and enrichment marker of low abundant signaling proteins that otherwise are not detectable by global proteome analysis. EVtrap can also be used for disease-related DNA/RNA analysis after complete capture of EVs. As a platform to discover, validate and routinely detect diagnostic markers, EVtrap approach offers enormous potential to find signaling proteins in biofluids that were not previously accessible.

Tymora is seeking collaboration partners interested in this technology for design and validation of early disease detection tests, cancer monitoring post-treatment, or for companion diagnostic assays for pipeline therapies.

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