Pressure BioSciences Inc.

PCT NEWS

OTCQB: PBIO
Discovery Starts With Sample Preparation™

Volume 9, Issue 8 August 2015

Pressure BioSciences Closes $2,180,000 Initial Tranche of a $5 Million Private Placement

Senior Secured Convertible Debenture Offering Closed at Market Price with Common Stock Warrants Exercisable at a 43% Premium; Proceeds Will Support Expansion of Marketing and Sales Team and Pay-down of Convertible Debt

Excerpts from Review Article Published in Biochimica et Biophysica Acta

Mass spectrometry-based proteomic quest for diabetes biomarkers

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South Easton, MA, July 23, 2015 – Pressure BioSciences, Inc. (OTCQB: PBIO) ("PBI" and the "Company"), a leader in the development and sale of broadly enabling, pressure cycling technology ("PCT")-based sample preparation solutions to the worldwide life sciences industry, today announced that it has closed on gross proceeds of $2,180,000 from the initial tranche of a $5 million Private Placement (the "Offering"). Of the amount invested, $1,520,000 was received in cash and $660,000 was from the conversion of principal and interest on outstanding promissory notes. One or more additional tranches in the Private Placement may close on or before August 7, 2015.

Pursuant to the Subscription Agreement, the Company will issue Senior Secured Convertible Debentures with a fixed conversion price of $0.28 per restricted common share, and Common Stock Purchase Warrants exercisable into a total of 3,892,857 shares of restricted common stock at an exercise price of $0.40 per share. The Company is under no obligation to file a registration statement to register the shares underlying the Debentures and Warrants.

The Company netted $1,340,000 in cash after taking into account fees related to the Offering and the value of the notes that converted into the Offering.

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UPCOMING EVENTS

Pressure BioSciences, Inc. (PBI) to showcase its new cutting-edge Barozyme HT48 high throughput instrument for enhanced enzymatic digestion of proteins and PCT-HD for both protein extraction and digestion from biopsy-sized tissue samples at the HUPO 14th Annual World Congress (HUPO 2015), September 28 – 30, 2015, Vancouver Convention Center, Vancouver, British Columbia, Canada

Excerpted from Biochimica et Biophysica Acta
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Mass spectrometry-based proteomic quest for diabetes biomarkers

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ABSTRACT
Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia, which affects hundreds of millions of individuals worldwide. Early diagnosis and complication prevention of DM are helpful for disease treatment. However, currently available DM diagnostic markers fail to achieve the goals. Identification of new diabetic biomarkers assisted by mass spectrometry (MS)-based proteomics may offer solution for the clinical challenges. Here, we review the current status of biomarker discovery in DM, and describe the pressure cycling technology (PCT).

Sequential Window Acquisition of all Theoretical fragment-ion (SWATH) workflow for sample-processing, biomarker discovery and validation, which may accelerate the current quest for DM biomarkers. This article is part of a Special Issue entitled: Medical Proteomics

INTRODUCTION
Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia. Individuals suffering from DM are estimated to increase worldwide from 171 million in 2000 to 366 million in 2030 [1]. There are two main subgroups of DM, type 1 (T1DM) and type 2 diabetes mellitus (T2DM) [2]. T1DM results from selective autoimmune damage to insulin-producing β cells which lead to irreversible dysfunction of the cells [3]. T2DM presents two major defects β-cell dysfunction and insulin resistance in peripheral tissues, resulting from various causes including glucose toxicity and lipotoxicity [4, 5]. Chronic hyperglycemia of diabetes can cause long-term damages to different organs, especially eyes (diabetic retinopathy), kidney (diabetic nephropathy), and nerves (diabetic neuropathy) [6]. The prevalence of diabetic complications rises up to 98% for patients diagnosed with diabetes for 10 years or more and the complications severely affect patient's quality of life and can ultimately lead to death [6, 7].

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Mr. Richard T. Schumacher, President and CEO of PBI, commented: "The priorities for the use of funds raised in the initial tranche are (i) to increase the number of personnel in our marketing and sales department, to continue building the installed base of instruments and utilization of consumables, and (ii) to retire a significant portion of the variable priced convertible debt we took on to help facilitate growth while additional equity capital was being raised. It is expected that cash received from any additional closings that might occur in the near future will be used to pay off any remaining variable priced convertible debt."

This press release is not an offer to sell or a solicitation of offers to participate in the Offering. The units, including the shares underlying the Debentures and Warrants, have not been registered under the Securities Act and may not be sold in the United States absent registration under the Securities Act or an applicable exemption from registration.

For more information on the Offering, please see the Form 8k filed by the Company on July 23, 2015.

About Pressure BioSciences, Inc.
Pressure BioSciences, Inc. ("PBI") (OTCQB: PBIO) develops, markets, and sells proprietary laboratory instrumentation and associated consumables to the estimated $6 billion life sciences sample preparation market. Our products are based on the unique properties of both constant (i.e., static) and alternating (i.e., pressure cycling technology, or PCT) hydrostatic pressure. PCT is a patented enabling technology platform that uses alternating cycles of hydrostatic pressure between ambient and ultra-high levels to safely and reproducibly control bio-molecular interactions. To date, we have installed over 250 PCT systems in approximately 160 sites worldwide. There are over 100 publications citing the advantages of the PCT platform over competitive methods, many from key opinion leaders. Our primary application development and sales efforts are in the biomarker discovery and forensics areas. Customers also use our products in other areas, such as drug discovery & design, bio-therapeutics characterization, soil & plant biology, vaccine development, histology, and forensic applications.

Forward Looking Statements
Statements contained in this press release regarding PBI's intentions, hopes, beliefs, expectations, or predictions of the future are "forward-looking" statements within the meaning of the Private Securities Litigation Reform Act of 1995. These statements are based upon the Company's current expectations, forecasts, and assumptions that are subject to risks, uncertainties, and other factors that could cause actual outcomes and results to differ materially from those indicated by these forward-looking statements. These risks, uncertainties, and other factors include, but are not limited to, the risks and uncertainties discussed under the heading "Risk Factors" in the Company's Annual Report on Form 10-K for the year ended December 31, 2014, in the Company's Quarterly Report on Form 10-Q for the period ended March 31, 2015, and other reports filed by the Company from time to time with the SEC. The Company undertakes no obligation to update any of the information included in this release, except as otherwise required by law.

For more information about PBI and this press release, please click on the following website link:

http://www.pressurebiosciences.com

Please visit us on Facebook, LinkedIn, and Twitter

Excerpted from Biochimica et Biophysica Acta Volume 1854, Issue 6, June 2015, Pages 519–527
Mass spectrometry-based proteomic quest for diabetes biomarkers: **Continued from Page 1, Column 2**

Although great advances have been achieved the field of diabetes research over the past decades, a multitude of clinical problems persist. The identification of new biomarkers for early diagnosis and prediction of complications, particularly those in easily accessible clinical samples, would be useful to improved clinic outcome. Herein, we review the current status of diabetic biomarker research and provide some insights into the limitations and possible solutions for biomarker discovery and validation.

Specimens in DM biomarker research

3.2. Tissue samples
It is well known that some tissue-specific proteins are highly diluted in blood and only represent marginal levels of the total plasma protein content [24]. Therefore, tissue samples should be more direct and appropriate although they are not easily accessible and always minute in size (such as biopsy samples for diabetic microangiopathy and nephropathy detection). Therefore, sample preparation needs to be improved and optimized for low-abundance protein analysis. Pressure cycling technology (PCT), based on the principle that programmed cycles oscillating between ultra-high and ambient pressure induces dissolution of matrix from sample, has recently been developed as a new technology to ameliorate the complex proteomic sample handling for minute sample amounts [41,42].

Our data showed that 0.2 – 0.5 mg human wet kidney tissue can generate about 20 μg peptides, which is enough for 20 injections of MS analysis (Fig. 2, manuscript in preparation). Additionally, proteomes generated from these minute samples are similar to that from large samples (2 – 3 mg), illustrating no information loss by sample amount limitation. With this technology, minute kidney biopsy may be analyzed for the biomarker study of diabetic nephropathy.

Biospecimens are commonly stored in the form of formalin fixed paraffin-embedded (FFPE) tissues and such samples represent a large collection of pathological samples worldwide. They are highly stable at room temperature, and hence an attractive sample resource, especially for retrospective biomarker studies. It is expected that valuable biomarkers from human pancreas FFPE sections could be identified. However, formaldehyde treatment and histological processing significantly reduce protein extraction efficiency and may lead to protein misidentification, which limit the use of FFPE tissues for proteomic analyses. Aged FFPE tissues are also hardened, making proteins difficult to be extracted and solubilized. With PCT-based sample process (heat augmented with high hydrostatic pressure), the number of non-redundant proteins identified in the FFPE tissue was nearly identical to that of matched fresh-frozen tissue, reported by Fowler and coauthors [43].

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Protein validation in diabetic biomarker research

Therefore, we can collect plasma, biopsy tissue samples, or FFPE sections (PCT-based preparation) for SWATH analysis from pre-diabetic status to advanced stages of this disease, so as to get digital maps for permanent storage without information loss. With the diabetes-related mass spectrometric reference maps established, researchers can directly perform targeted navigation of apriori selected clinically relevant proteins by S/MRM (Fig. 5). It is worth mentioning SWATH-MS, owing to its high reproducibility comparable to S/MRM, has the potential to be used as validation approach as well although it requires rigorous cross laboratory examination.

CONCLUSIONS

The incidence of DM is markedly increasing worldwide, which attracts more and more efforts on improving the clinical outcome for diabetic patients. Several clinical challenges remained to be overcome. The existing clinical markers fail to offer early diagnose or predict diabetes and its complications. MS-based proteomics stands out as a promising approach to explore and validate new diabetic protein biomarkers. In order to apply MS-based proteomic methods, proteins from biospecimens must be efficiently extracted and digested into peptides which are subsequently ionizable in the mass spectrometer. Plasma samples are the most favorite specimen for diabetic biomarker studies; however, efficient proteomic analysis of plasma is difficult due to the extra-high dynamic range of protein expression. Alternative specimens including tears, saliva, and fresh frozen/FFPE tissues also offer unique insight to the biomarker studies of diabetes and its complications. PCT is a promising sample preparation technology, which permits fast and robust proteomic sample preparation from minute amount of specimen with minimal technical variation. MS-based proteomics is evolving rapidly and has penetrated into many branches of biomedical research in recent years. Obsolete methods still frequently appear in literature of DM research. The targeted proteomics (SRM) and next generation proteomics (SWATH) offer transformed reproducibility, sensitivity, and proteome coverage. However, they have not been properly introduced into DM research although initiative attempt like HUPO B/D-HPP has started to change the situation.

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Pressure BioSciences' PCT-HD System (PCT High Definition)

A Tissue Homogenization, Protein Extraction and Digestion System for Biomarker Discovery and Mass Spectrometry Sample Preparation

Barocycler NEP2320-Enhanced
(45 kpsi-Elevated Temperature to 90°C)

MicroPestles and MicroTubes

PCT-HD Specification Sheet

Request Information

Features and Benefits

- Extract Proteins from Minute Tissue Biopsy Samples (0.2 - 3.0 mg of Soft Solid Tissue)
- Unprecedented Speed and Reproducibility with Pressure Cycling Technology (PCT)
- Rapid Digestion of Proteins (Minutes vs. Hours)
- Compatible with a Variety of Enzymes, including Trypsin, Lys-C, PNGase F, Chymotrypsin and Many Others
- Process Up to 16 Samples Simultaneously
- Inert, Single-Use MicroPestles and MicroTubes

PCT-HD was developed in collaboration with Dr. Ruedi Aebersold, his colleague Dr. Tiannan Guo, and PBI scientists and engineers. The Aebersold Lab incorporates the PCT-HD System as an integral part of *PCT-SWATH* in their research efforts.