PTMsense ™ - biosensor for an electrochemically-based point-of-care diagnostics

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ABSTRACT

Detection and profiling of ubiquitination patterns of individual cancer patients will allow the prediction of sensitivity to specific drugs as a means for precision medicine and improved care. PTM Biosciences is developing the PTMsense™; a novel microscale electrochemical biosensor for highly sensitive detection of ubiquitination in liquid biopsies. The biosensor will be further developed and integrated as the core technology of prognostic kits that will allow in a fast and accessible way, adjusting the treatment plan for each patient in point-of-care settings and hold the potential of improving the clinical outcome of cancer patients in parallel to decreasing healthcare costs.

Keywords: Ubiquitin, Redox, Ferrocene

1. INTRODUCTION

Today, medical practice is mostly based on a trial and error approach for treatment selection, often resulting in loss of critical time and unnecessary exposure to toxic drugs. This is especially critical in cancer where the percentage of responders to available drugs is low and even the newest drugs do not benefit the majority of patients. Due to suboptimal outcomes, the face of medical practice is undergoing a profound change by adopting precision medicine as an emerging approach for disease management and patient care. Precision medicine takes into account individual variability based on molecular biomarkers to determine which medical treatment will work best for each patient. Currently, precision medicine is based on genomic changes, including mutation analysis, mRNA and miRNA expression profiling, which are often limited since they report on rather indirect effects that frequently do not correlate with the actual physiological state.

Proteins are the functional units responsible for carrying out all biological processes and their activity is the ultimate determinant of physiological state. Protein activity is controlled by a set of chemical modifications termed Post-Translational Modifications (PTM). Specifically, modifications by ubiquitin (ubiquitination) regulate many processes in the cell such as protein degradation, activation/deactivation, protein trafficking and transcription regulation[1-3]. Ubiquitination plays a central role in regulating cellular function and maintaining homoeostasis in every cell, and indeed ubiquitination is involved in different aspects of cancer development and progression, such as dysregulation of cell growth and resistance to apoptosis. Despite their importance, due to the lack of enabling technologies the analysis of protein modifications and specifically ubiquitination is very limited and preventing their utilization in precision medicine.

Here we introduce a different approach of generating PTM-based molecular signatures that highly correlate with the physiological state and sensitivity to drugs, changing the focus of precision medicine from genomics to proteomics. In the scope of this project, we aim to develop a novel microscale electrochemical biosensor for highly sensitive detection of ubiquitination patterns on target proteins in liquid biopsies. The biosensor will be further integrated (outside the scope of this project) as the core technology of companion diagnostic kits that can be used in point-of-care settings to adjust the treatment plan for each patient, increasing the efficiency of the treatment[4]. The phase 1 focused on the biosensor electrochemically active analyte and the team has demonstrated the ability to generate a redox active ubiquitin derivative as a probe for ubiquitination.

2. STATE OF THE ART

Ubiquitin is a highly conserved small protein among all eukaryotes[1]. Ubiquitination involves the conjugation of the ubiquitin protein to many target proteins in the cell by dedicated ligase proteins. The labelling of target proteins with ubiquitin or poly ubiquitin chains is done by a cascade of enzymes designated E1, E2 and E3 that activate, carry and ligate it to the target. The conjugation of ubiquitin with its target occurs through one of the seven Lysine residues, the C' or the N' terminus[5].

Ubiquitin modified proteins are detected using conventional biochemical assays. Low throughput assays include Western blot analysis and ELISA. High throughput assays include protein arrays containing up to ~20,000 proteins printed on an absorbing surface. Labelling of the ubiquitin monomers is possible using ubiquitin derivatives labelled with fluorophores and other tags. However, these tags may hamper the ubiquitin activity due to blockage of the essential Lysin residues or the N-terminal of the protein. Available assays require primary and secondary antibodies against the ubiquitin chains or tags using chemiluminescence, colorimetric or fluorescent signals for detection. In addition, a designated expensive fluorescence scanner is required for data acquisition; the ubiquitin-modified proteins are detected and relatively quantified by the fluorescence scanner using the fluorescent signal. The use of fluorescent-labelled antibodies and fluorescence scanners to detect the ubiquitin-modified proteins complicates and hampers the utilization of protein modifications as companion diagnostic kits in point-of-care settings, thus limiting their commercial potential.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

We aim to develop the PTMsense™ as a novel microscale electrochemical biosensor for highly sensitive detection of ubiquitin modifications of target proteins in liquid biopsies of cancer patients. The biosensor will be further integrated (outside the scope of this project) as the core technology of a prognostic kit. Detection and profiling of ubiquitination patterns of individual cancer patients by the proposed point-of-care prognostic kit will allow, in a fast and accessible way, to predict sensitivity to drugs and adjust the treatment plan for each patient, thus facilitating precision medicine and improving the clinical outcome of cancer patients in parallel to decreasing healthcare costs.

The PTMsense™ is composed of the following two components:

- Biosensor platform, which includes a set of target proteins, captured on a microelectrode at a selective and desired orientation.
- Biosensor electroactive analyte, a novel ubiquitin derivative; redox labelled at a desired site.

These highly controlled components will enable electrochemical sensing of target proteins, which are modified by the redox labelled ubiquitin. The use of the redox active ubiquitin as a probe for ubiquitination will enable highly sensitive detection of protein ubiquitination patterns. The suggested electrochemical sensing approach offers multiple improvements over the traditional, laboratory-based fluorescence detection approach: (1) Superior signal to noise ratio and sensitivity; (2) Operational simplicity; (3) Portability, and (4) Eliminates the need for detecting antibodies and expensive fluorescence scanner.

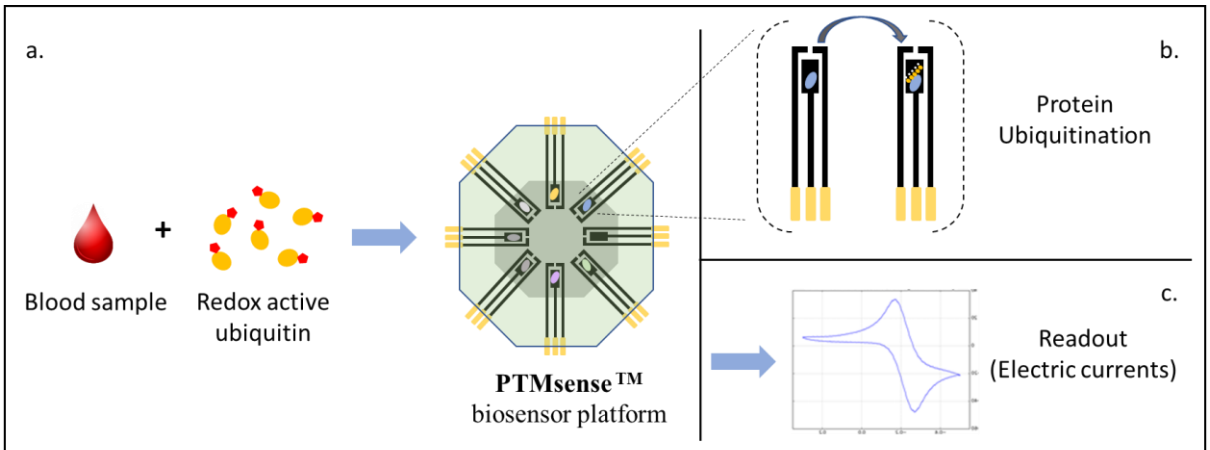

Fig. 1. PTMsense™ main utilization steps

4. PROJECT RESULTS

As part of this phase 1 project, we focused on the biosensor electrochemically active analyte. The team has demonstrated the ability to generate a redox active ubiquitin derivative as a probe for ubiquitination.and tested it on an initial setup representing the biosensor platform.
As an initial step we had to select target proteins and verify their ubiquitination levels. Four target proteins were selected and were shown to be substrates for ubiquitination reaction.

We then immobilized the target proteins on a solid support (multiplex microbead; Luminex Corporation) substituting the biosensor platform. The beads were functionalized with the appropriate ligands to be used also on an electrode surface. We also tested and compared oriented & non-oriented forms of the target proteins to assess the advantages of each form. The ubiquitination levels of the proteins were evaluated and compared. The results demonstrate advantage in using oriented proteins, as the ubiquitination levels of the oriented vs non-oriented proteins are higher.
The next step was the design and production of redox active ubiquitin derivatives serving as the biosensor active analyte. Following testing several concepts, two ubiquitin derivatives were proposed to allow the attachment of a redox molecule at specific positions using different chemistries. For the conjugation of redox molecules to the ubiquitin derivatives we have tested different chemistry approaches and verified that the redox conjugated ubiquitin derivatives were able to incorporate to target proteins.

Following analysis of the derivatives yield we have selected and prepared a Mono Ferrocene-ubiquitin that was purified and characterized (Fig. 2). The Mono Ferrocene-ubiquitin was successfully incorporated to a target protein (p53) immobilized on beads. The ubiquitin derivative was also compared to wild-type (WT) ubiquitin using a ubiquitination assay to verify that the modifications did not abolish the protein function (Fig. 3).

As for the electrical sensing, we used immobilized target protein on the surface of a screen printed electrode. The Ferrocene-labelled ubiquitin successfully ubiquitinated the target protein on the surface of a screen printed electrode. Our preliminary results showed an electrochemical signal specific to the incorporated Ferrocene-labelled ubiquitin (Fig. 4). Non ubiquitinated protein and protein ubiquitinated with wild type ubiquitin (with no Ferrocene) showed no electrochemical signal.

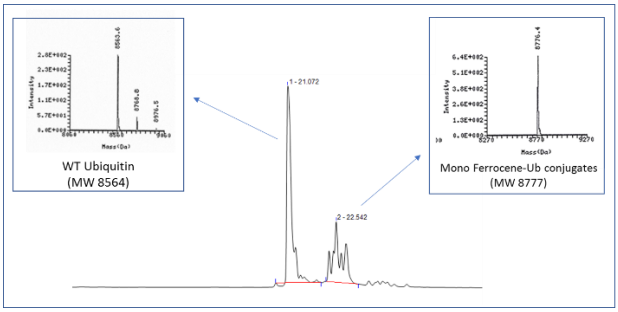

Fig. 2. Ferrocene-labelled ubiquitin was prepared, purified and characterized, to serve as the Biosensor electroactive analyte.

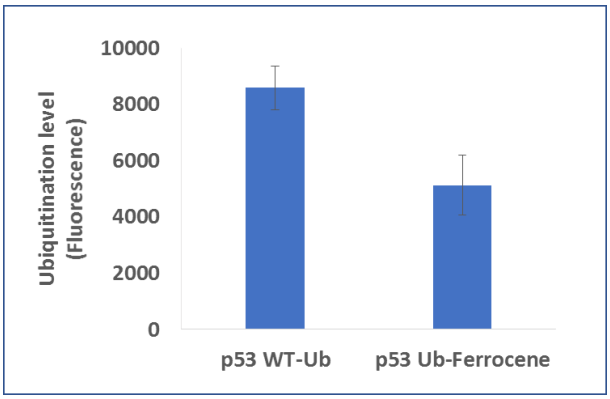

Fig. 3. Ferrocene-labelled ubiquitin was comparably active as wild type (WT) ubiquitin in modifying target protein (p53).

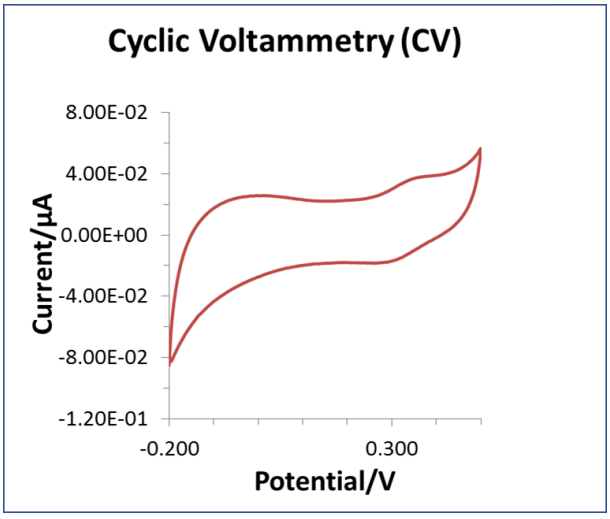

Fig. 4. Ferrocene-labelled ubiquitin showed electrochemical signal when attached to electrode.

5. FUTURE PROJECT VISION

5.1. Technology Scaling

We aim to develop prognostic kits that will allow in a fast and accessible way in point-of-care settings the prediction of sensitivity to certain drugs as a means to facilitate precision medicine for cancer patients.
During the Phase 2 we will continue the development of the PTMsense™ electrochemical biosensor main components:

- Selection of an indication (a specific cancer type), for which there is already an approved drug, but there are no known biomarkers associated with sensitivity to that drug.
- Identify a novel set of putative biomarkers (target proteins) able to predict sensitivity to the specific drug in the selected indication, based on a screening phase utilizing PTM Biosciences' post-translational modification profiling platform.
- Biosensor assay prototype development. The assay will incorporate the novel set of target proteins.
- Biosensor chip design and development. The chip will allow readout and analysis of the biosensor assay electrical signals.
- Conduct a pilot study in clinical settings to demonstrate the biosensor sensing and interpretation capabilities.

In the longer term and based on the electrochemical biosensor developed, additional kits can be developed and commercialized for different cancer indications.

5.2. Project Synergies and Outreach

In order to achieve the Phase 2 goals and continue the development of the PTMsense™ the Consortium will be expanded to include the following additional partners:

- Leading clinical sites will provide access to clinical samples and clinical expertise. We have ongoing collaborations with leading medical centers and frontline clinicians and researchers.
- Leading technology partners will provide product development expertise and know-how regarding electrochemical signal processing and chip design.
- Specialty manufacturers will provide protein expression and purifications services to produce the set of target proteins integrated as part of the biosensor.

The Phase 2 results including the pilot study findings will be disseminated in scientific publications and conferences.

5.3. Technology application and demonstration cases

Demonstrating the PTMsense™ capabilities during the Phase 2 will bring the following benefits:

- Clinical - the utility of the PTMsense™ as a prognostic kit with means to provide precision medicine in cancer with the potential to improve the clinical outcome in parallel to decreasing healthcare costs.
- Scientific - the PTM-based electrochemical biosensor will foster research and uncover the potential of protein modifications to reveal the actual physiological and disease states and provide key information for identification of novel drug targets, elucidation of mechanism of action of drugs, and discovery of predictive biomarkers.
- Industrial - a novel microscale electrochemical biosensor for highly sensitive detection of protein modifications will create an opportunity for new markets and growth engines with numerous applications in Healthcare and may be expanded into Agriculture, and Veterinarian domains.

Benefits to the Research Infrastructure communities in Europe include

- Knowledge sharing and unique access to cutting edge expertise in R&D activities, product development and commercialization;
- Attract foreign investment in Europe and its industry;
- Attract skilled professionals to collaborate with European projects and companies.

5.4. Technology commercialization

In parallel to the Phase 2 tasks of further technical development and clinical validation of the PTMsense™, and as a preparation for commercialization we will conduct several tasks as part of the commercialization process:

- Market research to gain comprehensive insights in regards to the relevant market segments, business model, penetration strategy, pricing, distribution etc.

- Partner with leading drug companies in order to gain access to discovery and research platforms of novel drugs and support market penetration.
- Protect the new intellectual properties (IP) and know how expected to generate as part of the project.
- Execute regulatory plan to obtain regulatory approvals and CE Mark.
- Scale up manufacturing towards product launch.

The ATTRACT Phase 2 program perfectly aligns with the project plan, and will provide us with the essential resources and guidance for the technical and clinical development activities, and the commercialization process of the PTMsense™. Commercially, the PTMsense™ represents a revolutionary solution to address a significant unmet clinical need and tap on the precision medicine emerging market expected to grow exponentially, generating a solid growth engine for PTM Biosciences and the European community.

5.5. Envisioned risks

The ATTRACT Phase 2 development project of the PTMsense™ is a highly innovative and entails several technological and commercial risks. The main challenges and mitigation avenues:

- Electrochemical chip design and performance: We will partner with leading technology players to gain access to product development expertise and know-how regarding electrochemical signal processing and chip design
- Marketing restraints, including obtaining regulatory approvals (CE Mark and others) and ensuring reimbursement criteria, claim coding, and billing procedures, may prolong the commercialization of the PTMsense™.: We will employ as part of the Phase 2 expert regulatory and reimbursement advisors to define a comprehensive regulatory and reimbursement strategies and plans to ensure the successful commercialization of the PTMsense™.
- Market penetration and adaptation: We collaborate with clinical luminaries in the field of Cancer to produce solid, validated clinical data. In addition, we will form collaborations with leading players in the market, in order to utilize their existing marketing and distribution channels.
- Competition: Developing a prognostic kit requires deep understanding and expertise, as well as special infrastructure. we will employ as part of the Phase 2 expert IP advisors and protect the new IP and knowhow generated as part of this project which will serve as entry barriers for potential competitors.

5.6. Liaison with Student Teams and Socio-Economic Study

As part of the Phase 2 we will generate MSc. level explanation materials of the PTMsense™ underlying technologies including the profiling of post-translational modifications and the electrochemical sensing.

We will provide information to the socio-economic study of the ATTRACT initiative and ecosystem by joining interviews with study researchers and attending related conferences and meetings.

6. ACKNOWLEDGEMENT

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