PREECLAMPSIA RISK STRATIFICATION EARLY IN PREGNANCY: LEVERAGING A PROMISING METABOLOMICS DISCOVERY INTO A LC-MS BASED CLINICAL ASSAY

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Abstract

Basic metabolomics research has uncovered that combinations of blood borne metabolites can triage women early in pregnancy according to their risk of developing preeclampsia[1].

Since then, a company has been established which is dedicated to translating this finding into a tool for health care providers and pregnant women.
A targeted approach is being developed whereby metabolites of interest are (semi-)quantified using liquid chromatography-tandem mass spectrometry.

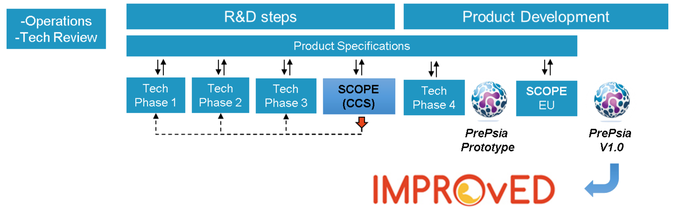
Next, large scale verification and validation studies within dedicated prospectively collected biobanks will be performed to progress this multimarker assay and prediction algorithm.

Introduction

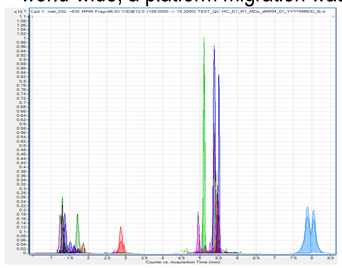
- Thus far preeclampsia risk prediction is mostly depending on clinical risk factors, yet this risk prediction is inadequate for healthy first time pregnant women. With first time pregnancies accounting for >50% of all preeclampsia cases, there is a clear need for dedicated prediction tools.
- The complex pathogenesis of the syndrome, warrants for a panel of biomarkers in order to predict preeclampsia in first time pregnant women.
- Kenny *et al*[1] from the University College Cork, Ireland found that accurate prediction of preeclampsia in 1st time pregnant women is possible using a panel of blood-borne metabolite present in plasma of ~15 weeks pregnant women.
- Metabolite analysis using a liquid chromatography-mass spectrometry platform (LC-MS) is well established in pharma (bioanalysis) and neonatal screening ("heel prick").
- A company was founded to further develop a metabolite-based preeclampsia risk stratification test on an LC-MS platform to deploy in clinical laboratories.
- A prototype LC-MS methodology has already been developed and is ready for application in clinical samples.
- Successful development of diagnostics requires access to intended population samples: public-private partnerships are in place with 2 large scale international prospectively collections of first-time pregnancy biospecimens, i.e. SCreening fOr Pregnancy Endpoints (SCOPE[2]) and IMproved Pregnancy Outcomes by Early Detection (IMPROvED[3]).

Methods

1. A product development plan was produced outlining the necessary technology development and validation phases.



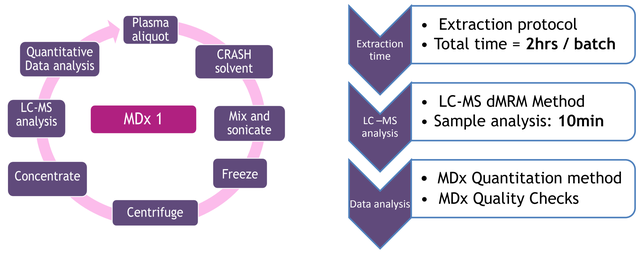
2. **Tech Phase 1**: The original biomarker discovery study relied on metabolite profiling using a high-end LC-MS set-up. To accommodate the large install base of quadrupole mass spectrometers (QqQ-MS) in clinical laboratories world-wide, a platform migration was necessary.



LC:
10 minute gradient elution. Agilent PFP Pursuit column; separation of hydrophilic and hydrophobic compounds
MS:
Dynamic Multiple Reaction Monitoring
2 transitions per analyte/internal standard:
112 MRM transitions monitored per run

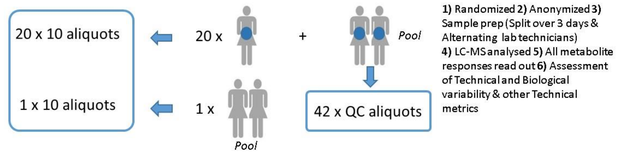
A multiplex LC-MS analysis was developed for 40+ target metabolites (Kenny et al[1]). Analysis is achieved in 10 minutes.

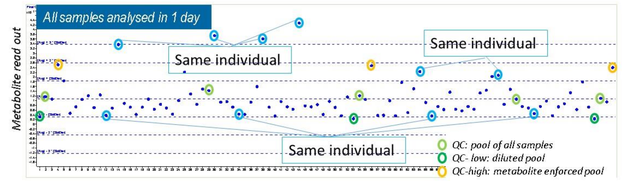
3. **Tech Phase 2**: For application into routine clinical laboratory practice a simple, reliable, automatable and cost-efficient sample preparation is required. This is integrated in a full analysis pipeline.



- A 10 min multiplex LC-MS analysis was developed for 40+ target metabolites[1].
- High precision achieved: 90% of metabolite assays have a %CV <15%
- Current throughput 405 clinical samples / week / platform.

3. **Tech Phase 3**: Prior to subjecting precious biobank samples analysis, the analysis pipeline is field-tested on separate plasma samples obtained from pregnant women at 15 weeks.

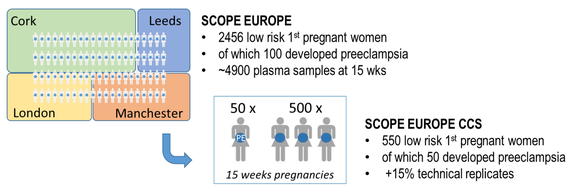




Robust, reproducible analysis of metabolites relevant to the prediction of preeclampsia in samples derived from the target population was achieved.

Next Steps

4. **CCS:** In Collaboration with the SCOPE consortium, a Case Control will be conducted by end of 2014. This study is geared to metabolite candidate confirmation and prediction algorithm refinement.



5. **Tech Phase 4:** A prototype of the multiplex assay and risk prediction algorithm will be put in place.
6. **SCOPE Europe cohort study:** Technical pre-validation of the clinical assay and verification of the prediction algorithm in the SCOPE Europe cohort.
7. **Product validation:** Full Technical and clinical validation of PrepSia v1.0 in IMPROvED (n ~5000)[3].

Conclusions

Within Metabolomic Diagnostics key translational research steps are already taken to propel a promising metabolite panel for preeclampsia risk prediction early in pregnancy closer to the clinical arena.

All major components are in place to commence processing the clinical samples as available in SCOPE and IMPROvED biobanks.

[1] Kenny, L. C. *et al. Hypertension* **56,** 741-9 (2010); [2] North, R. A. *et al.* t. *BMJ* **342,** d1875 (2011); [3] Navaratnam, K. *et al. BMC Pregnancy Childbirth* **13,** 226 (2013)

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The main goal of IMPROvED is to develop a clinically robust predictive blood test for pre-eclampsia, using innovative technologies and utilising novel metabolite and protein biomarkers. www.fp7-improved.eu

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