IMPROvED

Improved Pregnancy Outcomes by Early Detection

FINAL PROJECT REPORT

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Project acronym: IMPROvED

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LIST OF ABBREVIATIONS

AUROC	Area Under the curve of the Receiver Operating Characteristic
CCS	Case Control Study
CLSI	The Clinical & Laboratory Standards Institute
EAB	Ethics Advisory Board
eCRF	electronic Case Report Form
ESBB	European Society for Biopreservation and Biobanking
EU	European Union
FP7	Seventh Framework Programme
GCP	Good Clinical Practice
HPLC	High-performance liquid chromatography
ICH-GCP	The International Conference on Harmonisation-Good Clinical Practice
ICER	Incremental Cost-Effectiveness Ratio
IGFALS	Insulin-like growth factor-binding protein complex acid labile subunit
IMPROvED	IMproved PRegnancy Outcomes by Early Detection
IP	Intellectual Property
ISBER	International Society for Biological and Environmental Repositories
ISF	Investigator Site File
ISO	International Organisation for Standardization
IVD	In Vitro Diagnostics
KOL	Key Opinion Leaders
KU	Keele University
LCMS	liquid chromatography hyphenated with mass spectrometry
LDA	Low Dose Aspirin
MCAM	Melanoma cell adhesion molecule
NALA	National Adult Literacy Agency
NCD	Non-Communicable Diseases
NPV	Negative Predictive Value
PAB	Patient Advisory Board
PI	Principal Investigator
PlGF	Placental growth factor
PPV	Positive Predictive Value
ROC	Receiver Operating Characteristic
SA-PE	Streptavidin Phycoerythrin
SCOPE	SCreening fOr Pregnancy Endpoints
sENG	soluble Endoglin
SME	Small Medium Enterprise
SMF	Study Master File
SOP	Standard Operating Procedures
SPINT	Serine Peptidase inhibitor Kunitz type 1
UCC	University College Cork
UK	United Kingdom
US	United States
VC	Venture Capital
WHO	World Health Organisation
WP	Work Package
yr	Year

PROJECT PARTNERS

	University College Cork **UCC**
	accelopment AG **ACCEL**
	Erasmus Universitair Medisch Centrum Rotterdam **EMC**
	Karolinska Institutet **KI**
	Keele University **KU**
	MedSciNet AB **MEDSCINET**
	Metabolomic Diagnostics Limited **METABOL**
	MyCartis NV **MyCartis**
	Region Hovedstaden **REGIONH**
	Rijksuniversiteit Groningen **RUG**
	University of Liverpool **ULIV**

1 SECTION 1 – FINAL PUBLISHABLE SUMMARY REPORT

1.1 Executive Summary

The IMPROvED (IMproved PRegnancy Outcomes by Early Detection) project is a multicentre clinical study aiming to assess and refine innovative prototype screening tests for pre-eclampsia, a common complication of late pregnancy. Approximately 50 million babies are born to first time mothers worldwide every year, and almost 1 in 20 of these pregnancies are complicated by pre-eclampsia. The condition is associated globally with 70,000-80,000 maternal and over 500,000 infant deaths annually. For the mother, it can lead to acute problems in the liver, kidneys, brain and the clotting system

Identification of women at risk of pre-eclampsia is the first step to effective intervention and prevention. Current screening is based on the presence of clinical features. However, the majority of women who develop pre-eclampsia are first-time mothers, who commonly have no identifiable clinical risk factors in early pregnancy.

Prompted by the current absence of a clinically useful screening test for pre-eclampsia, the IMPROvED consortium aimed to develop a robust predictive blood test suitable for use in a clinical environment, employing innovative technologies and utilising novel metabolite biomarkers. Project partner Metabolomics Diagnostics (Ireland) had developed a prototype screening test (MetTest) for pre-eclampsia which was to be further refined and validated in IMPROvED, with the aim of ultimately progressing to regulatory approval and clinical use.

4063 first time mothers were found to be eligible and consented into the study in four European countries, Ireland, United Kingdom, the Netherlands and Sweden. Of those, 4005 completed the study by attending at least two appointments at 15 and 20 weeks' gestation. A subset of these attended up to four visits, with additional optional time points at 9-11 weeks' and/or 32 weeks' gestation, with an IMPROvED midwife. At each visit, comprehensive clinical data, blood, urine and hair samples were collected. A customised IMPROvED Clinical Data and Biobank Management Database, specifically designed for data management in clinical trials and cohort studies was developed by project partner MedSciNet. In parallel to participant recruitment and validation of the MetTest, project partner University of Groningen in the Netherlands, provided expertise to support the assessment of the health economic benefits of screening tests.

Metabolomic Diagnostics has finalised its translational research work and is now preparing its test, PrePsia™, for clinical validation with biobanked samples collected during the IMPROvED study. Metabolomic Diagnostics has subsequently been awarded €2 million in EU Funding under the latest Horizon2020 SME Instrument call in March 2018, as part of a plan to introduce PrePsia™ into the European market and beyond.

1.2 Summary Description of Project Context and Objectives

The IMPROvED (IMproved PRegnancy Outcomes by Early Detection) project aimed to assess and refine two innovative prototype screening tests for pre-eclampsia, a common complication of late pregnancy. Identification of women at risk of pre-eclampsia is the first step to effective intervention and prevention. Current screening is based on the presence of clinical features, however, the majority of women who develop pre-eclampsia are first-time mothers, who commonly have no identifiable clinical risk factors in early pregnancy. Approximately 50 million babies are born to first time mothers worldwide every year and almost 1 in 20 of these pregnancies are complicated by pre-eclampsia.

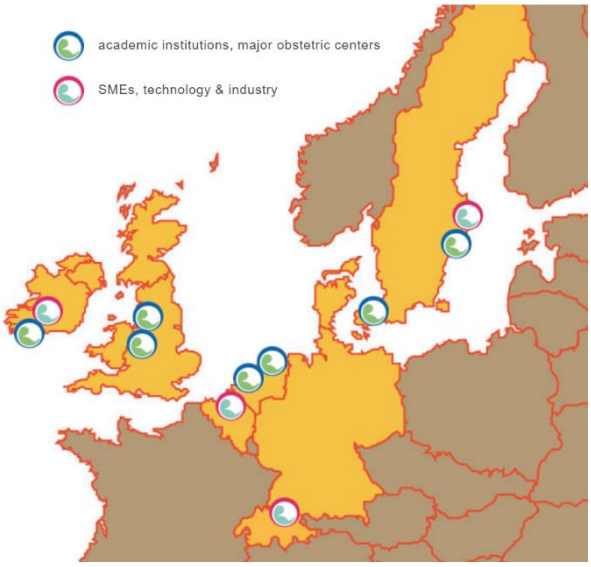


Figure 1: IMPROvED Project Partners.

The condition is associated globally with 70,000-80,000 maternal and over 500,000 infant deaths annually. For the mother it can lead to acute problems in the liver, kidneys, brain and the clotting system, and remains a common cause of iatrogenic prematurity.

The IMPROvED consortium consisted of 4 SME partners, Accelopment: Switzerland; MedSciNet: Sweden; Metabolomic Diagnostics: Ireland; and MyCartis: Belgium and 7 academic partners University College Cork, Ireland; University Medical Centre Rotterdam (Erasmus MC); Karolinska Institutet: Sweden; Keele University: United Kingdom; Region Hovedstaden (Copenhagen Trial Unit): Denmark; University of Groningen: Sweden; and University of Liverpool: United Kingdom (Figure 1).

The main goal of the IMPROvED study was to develop a clinically robust predictive blood test for pre-eclampsia.

The specific objectives were to:

i) recruit 5000 first-time pregnant women;

ii) determine whether prototype predictive assays and algorithms translate to the clinical environment;

iii) assess potential synergy of a combined metabolomic and proteomic approach;

iv) progress regulatory approval and development of the selected test into the clinical arena;

v) establish a residual biobank that can be accessed by the scientific community for high quality research into the cause and prevention of adverse pregnancy outcome.

Prompted by the current absence of a clinically useful screening test for pre-eclampsia, the IMPROvED consortium aimed to develop a robust predictive blood test suitable for use in a clinical environment, employing innovative technologies and utilising novel metabolite and protein biomarkers. Project partners Metabolomics Diagnostics (Ireland) had developed a prototype screening test (MetTest) for pre-eclampsia which was to be further refined and validated in IMPROvED, with the aim of ultimately progressing to regulatory approval and clinical use. The second project partner, MyCartis (Belgium), did not progress with their initial studies and halted their screening test development and validation work.

In IMPROvED, 4005 first time mothers participated in the study by attending at least two, and up to four, visits with an IMPROvED midwife. Initially recruitment was planned in five European countries; Ireland, U.K., Germany, the Netherlands and Sweden. However, the study was not feasible in the German site, and they subsequently withdrew from the study at an early stage. At each visit comprehensive clinical data, blood, urine and hair samples were collected. A customised IMPROvED Clinical Data and Biobank Management Database, specifically designed for data management in clinical trials and cohort studies, was developed by project partner MedSciNet. In parallel to participant recruitment and validation of the ProTest and MetTest, project partner University of Groningen, the Netherlands planned to assess the health economic benefits of screening tests.

IMPROvED has established a high calibre pregnancy biobank, accessible to pregnancy researchers which houses samples derived from women attending, at up to four time-points, multiple clinical centres during pregnancy and therefore are representative of different healthcare models. An appropriate governance plan in compliance with ethical and data protection regulations has been developed during the project. Figure 2 shows sample movement throughout the project; from collection and processing at each recruitment centre, to shipment of samples to UCC where sample reconfiguration has been performed allowing for a redistribution of a subset of up to one third of samples to Metabolomics Diagnostics. The remaining two thirds of the samples are housed in UCC forming the IMPROvED residual pregnancy biobank.

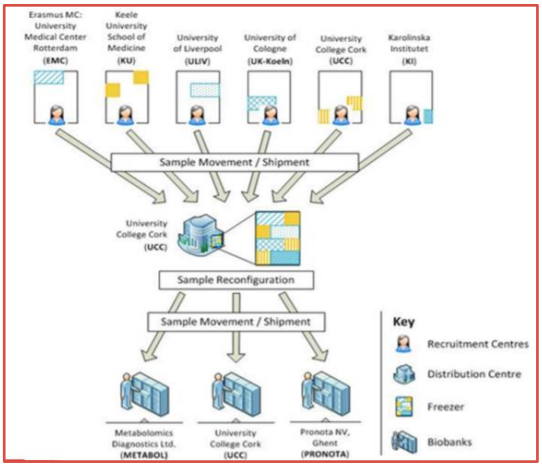


Figure 2: IMPROvED Biobank.

During IMPROvED, 4063 first time mothers who were found to be eligible consented to the study in four European countries, Ireland, U.K., the Netherlands and Sweden. Of those, 4005 attended the study for at least two appointments at 15 and 20 weeks' gestation and in some cases up to four visits with additional optional time points at approximately 11 and 32 weeks' gestation, with an IMPROvED midwife.

A lower number (n=4063) of participants than planned were recruited to the study, which reduced the projected power by approximately 20%. The estimated incidence of pre-eclampsia in the IMPROvED cohort was approximately 3%, but this is expected to increase as the International Society for the Study Hypertension in Pregnancy have redefined pre-eclampsia to include evidence of placental dysfunction. As a result, many participants with gestational hypertension will be reclassified as pre-eclampsia and this examination of participants is currently ongoing. This change is expected to increase the proportion of participants with pre-eclampsia, which, according to the new definition would be 5% to 7% rather than 3%.[1 2]

Our SME project partner, Metabolomic Diagnostics, has finalised its translational research work and is now preparing its test, PrePsia™ for clinical validation within the context of the biobanked samples from the IMPROvED study. Metabolomic Diagnostics has subsequently been awarded €2 million in EU Funding under the latest Horizon2020 SME Instrument call in March 2018 as part of a plan to introduce PrePsia™ into the European market and beyond.

1.3 Main Science &Technology Results/Foregrounds

The main goal of the IMPROvED project was to develop a clinically robust predictive blood test for pre-eclampsia. To fulfil this aim the IMPROvED Project held at its core a clinical study that aimed to recruit 5000 first-time pregnant women and biobank clinical samples from multiple time points across their pregnancies (WP03, Figure 3). The biobanked samples would provide validation sets for both SME partners; Metabolomics Diagnostics and Pronota (now MyCartis), and produce a residual biobank that could be accessed by the scientific community for high quality research into the cause and prevention of adverse pregnancy outcome.

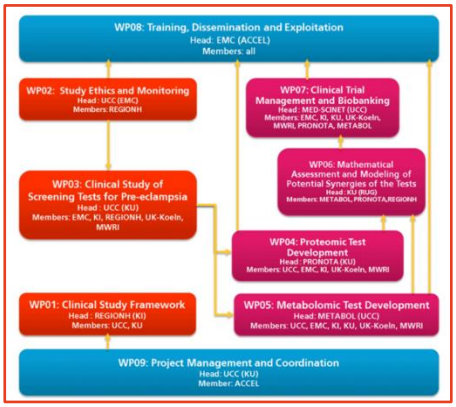


Figure 3: IMPROvED Project.

The IMPROvED Consortium will support the SME partners to determine whether their prototype predictive assays and algorithms translate to the clinical environment, and to progress regulatory approval and development of the selected test(s) into the clinical arena. Additional work will assess the Health Economics of the prototype predictive assay.

1.3.1 Standard Operating Procedures

The biobanked samples provided by the IMPROvED study will be used to validate the prototype predictive assays and algorithms. To ensure compliance with regulations, the IMPROvED Consortium recognised the importance of standardising processes across recruitment centres, in order to harmonise activities for this multi-centre clinical study. As the IMPROvED clinical database and biobank was pivotal to the project objectives, quality was maintained throughout the study from initial patient recruitment, to clinical data and biospecimen collection, biospecimen processing and storage, sample shipments and biobank governance. To ensure this, the IMPROvED project developed a set of Standard Operating Procedures (SOPs), which, in combination with IMPROvED specific training, aimed to promote quality and consistency in the project.

High quality, clear and detailed study specific SOPs were essential to ensure the consistent day to day performance of the clinical study. IMPROvED developed six SOPs, which were subject to review by the Consortium and subsequent approval by the Quality Committee. These SOPs comprised of:

- Midwifery
- Safe Working with Blood, Immunisation & Personnel Accident Reporting
- Biospecimen Collection & Transport to Laboratory
- Biospecimen Processing & Storage
- Freezer Management
- Data Monitoring

By necessity, some SOPs were more complex than others, for example, the Midwifery SOP outlined in detail each step from the potential participant identification or self-referral, through to the collection, entry and query of the clinical data. Given the importance of the Biobanking element of this study, separate SOPs were devised for Biospecimen Collection & Transport to Laboratory, Biospecimen Processing and Storage and Freezer Management. Additional Project SOPs such as the IMPROvED Internal Risk Assessment SOP were also developed.

1.3.2 IMPROvED Clinical Study Ethics

The IMPROvED study was conducted in accordance with ethical principles, sound scientific evidence and clear detailed protocols. The International Conference on Harmonisation-Good Clinical Practice (ICH-GCP) is an international ethical and scientific quality standard for the design, conduct, performance, monitoring, auditing, recording, analysis and reporting of clinical trials. In compliance with GCP guidelines, the study management team and REGIONH established the study master file (SMF) for IMPROvED. The documents included in the SMF will serve to demonstrate the compliance of the sponsor and investigators with applicable regulatory requirements including GCP, and will therefore, be able to support the regulatory approval and development of the selected test(s) into the clinical arena. A SMF and study site specific Investigator Site File (ISF) was in place prior to recruitment of the first participant. The SMF and ISF, which could only be finalised once the recruitment target was reached and all follow up data were entered into the IMPROvED database, and the investigators at each site have reviewed the SMF/ISF for completeness, and attested to the conduct of the study and the quality of the data collected.

In compliance with the Quality Plan, all staff involved in the IMPROvED project were fully trained, commensurate with their job description and work activity, to ensure critical tasks were only performed by qualified, trained personnel. An IMPROvED specific protocol, ethics and good clinical practice (GCP) training module was developed and approved by study management, and a training webinar was conducted (Figure 4).

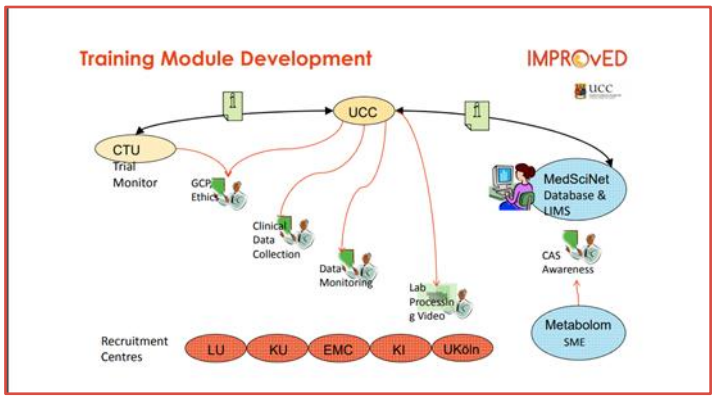


Figure 4: Training Module Development.

To further comply with the Quality Plan, quality standard evaluations and internal auditing were conducted in order to document, assure, and improve the clinical research performance. The feasibility of study participation at a clinical recruitment centre was aided, evaluated and documented in pre-study initiation reports. The five IMPROvED recruitment centres were visited prior to study participant enrolment, with all passing their site initiations (Figure 5). Completed site initiation reports were followed up, to assure tasks and issues were responded to appropriately. Centres were, throughout the study, further monitored in accordance with the monitoring plan.

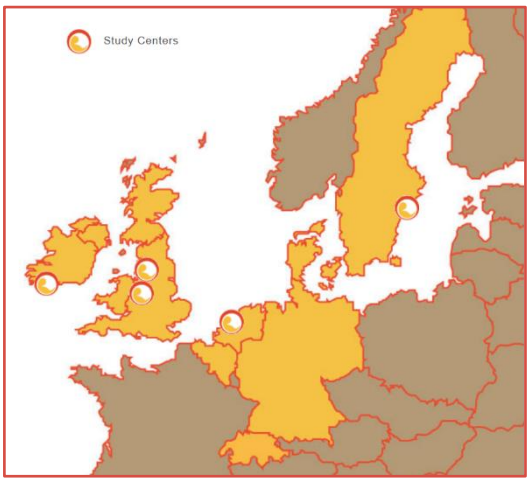


Figure 5: IMPROvED Sites.

The clinical study protocol was developed by UCC and KU with input from partners prior to the project start. It was originally proposed that the Ethics Advisory Board (EAB) would review the clinical protocol, however, it was decided to make use of the internal expertise in the consortium (REGIONH) for protocol review and to optimise input from the EAB by changing the focus of their input to ethical issues, in particular the governance of the residual biobank.

In compliance with the Quality Plan, ethical approval for the IMPROvED study was sought for each recruiting centre. Ethics was approved for 6 of the 7 proposed IMPROvED clinical recruitment sites, following which, enrolment of study participants commenced at these sites. Thus, these centres fulfilled national legal and ethical requirements for research on this vulnerable population. The ethical approvals obtained were continuously monitored throughout the study for validity and appropriateness, in a joint effort by the local PIs and the Study Ethics and Monitoring

Work Package, to ensure that they were compliant with protocol and other amendments. Where necessary, investigators liaised directly with local ethics committees to clarify any areas of ambiguity, and to determine that ethical approval was granted not only for the IMPROvED clinical study, but also for the retention of samples in the IMPROvED biobank. This, and the fact that the IMPROVED Study population is classified as vulnerable, added complexity to the Ethical Application and Approval processes. Karolinska University in Sweden was the first site for which Ethical Approval was obtained for on the 29th of April 2013, with the last being UK-Koeln, on the 19th of November 2014.

Although this process was time and labour intensive, and delayed the initiation of participant recruitment, the outcome has added to the overall quality of the IMPROvED study. Additional work on securing ethical approval for the IMPROvED study laid down the principles for the future governance of the biobank. The ethical approvals obtained for each clinical site outline the conditions for how the collected samples and data would be stored and used after the end of the IMPROvED project. Ethical documents such as Patient Information Leaflets (PILs) and Consent forms were created. To ensure that these and all ethics documents meet international plain English standards comprehensible by all literacy stages, they were verified by the National Adult Literacy Agency (NALA) in Ireland. The documents were then translated into the language used at each recruitment centre. These translations were then back-translated into English to ensure no loss of meaning had occurred.

As a result of the care taken to meet ethical obligations, the IMPROvED project has generated a residual and highly valuable biobank of clinical data and biological samples of different specimen types (including potential DNA sources) which is housed within an existing biobank facility at UCC.

For the patient recruitment process, two types of printed promotional material were designed: poster and flyer. They aimed to raise the women's awareness about the importance of their participation in the study; hence the attention-grabbing headline: “YOU CAN HELP MAKE PREGNANCY SAFER”. Poster and flyer were adapted to the local languages of the recruitment centre, hence there are in total four language versions for each product: English, Dutch, German and Swedish. The overall design and the headline (in English) remain uniform across languages. In all recruiting centres, the poster was displayed in highly visible areas, such as hospital entrances, waiting lobbies and amenities to maximise the visual reminders to patients.

On the 29th of November 2013, the first participant was recruited in Cork University Maternity Hospital (CUMH), a partner institute of IMPROvED Consortium member University College Cork (UCC). On the 3rd of March 2014 Royal Stoke University Hospital (Keele University) opened for recruitment, closely followed by University Medical Center Rotterdam (Erasmus MC Rotterdam) on the 12th of March 2014 and Karolinska University Hospital Huddinge, Stockholm (Karolinska Insitute) on the 22nd of April 2014. Liverpool Women's Hospital (University of Liverpool) began recruitment on the 4th March 2014 and had recruited their 100th participant by the 30th of September 2014.

On December 21st 2016, the last participant was recruited to IMPROvED in Rotterdam, bringing the total participants recruited at this site to 818. The final IMPROvED participant in Cork was recruited on the 3rd of August 2017, bringing to 4063 the number of women recruited to the IMPROvED Study across 5 centres in 4 countries.

UK-Koeln had planned to recruit patients at both Uniklinik Koln, (Cologne) and Universitats Kilnikum, Bonn. For efficiency, and based on local experience, it was decided to obtain ethical approval and begin recruitment in Cologne before applying for ethical approval in Bonn, with the hope being that this would ease the subsequent application process in Bonn. In addition, a third-party contract would be required for Cologne and Bonn. Recruitment commenced in Cologne, but after the eventual recruitment of 5 participants, it was decided that UK-Koeln would terminate their participation in the IMPROvED consortium in 2015. None of the 5 participants recruited in UK-Koeln completed the study.

1.3.3 Ethics Advisory Board

Scientific Lead Prof Louise Kenny initiated the IMPROvED Ethics Advisory Board (EAB). It consisted of three members from contrasting backgrounds; Law, Child Health and Obstetrics, each of whom had extensive expertise in their own area. An EAB Charter was drawn up by the IMPROvED Project Manager and signed by each EAB member. It had been originally proposed that the Ethics Advisory Board (EAB) would review the clinical protocol, however, it was decided to instead optimise input from the EAB by directing the focus of their input to ethical issues, in particular the governance of the residual biobank. The Ethics Advisory Board Meeting took place on 24th April 2018. During this meeting, the EAB were given an overview of the project by the Principal Investigator and an update of study conduct by the Project Manager, and they provided recommendations on the future governance of the residual biobank and the implementation of the new IMPROvED consortium agreement which will post-date the end of the IMPROvED Project Grant.

1.3.4 IMPROvED biobank

Establishing and maintaining a biobank is complex and involves different areas, including; ethics, regulatory, scientific/technical, economical, etc. The IMPROvED Consortium recognised the complexity and importance of establishing a residual IMPROvED biobank, and the importance of ethical and regulatory considerations around biobanking.

The IMPROvED Consortium has recognised the importance of training to allow a highly professional biobank to evolve (Figure 2). As the IMPROvED biobank is pivotal to the project objectives, maintaining quality throughout the study was paramount; from initial patient recruitment, to clinical data and biospecimen collection, biospecimen processing and storage, sample shipments and biobank governance. Emphasis was placed on complying with the highest standards in ethics, data protection, Good Clinical Practice (GCP) and best international practices in biobanking. The creation of a toolbox of training aids and materials and a commitment to training delivery has ensured IMPROvED personnel are trained to a level resulting in standardisation of all processes across all recruitment centres. Training modules for clinical and technical staff were also developed in compliance with the strict monitoring procedure developed for, and communicated within, the project.

The IMPROvED Consortium has outlined a governance structure for residual IMPROvED biobank and developed strict procedures for data protection of IMPROvED participants and storage of collected biological material. The EAB has agreed to continue to review any ethical issues and has undertaken the design and review of the residual biobanking governance. A Steering Committee will act as a custodian for the residual biobank after the project completion, and a formalised process for access to the IMPROvED biobank has been outlined.

There are over 80 best practice guidelines applicable to biobanking and in December 2013, the International Organisation for Standardization (ISO) established a Technical Committee for

Biotechnology (TC 276), to develop the first set of international standards for the biotechnology industry. The ISO Technical Committee for Biotechnology is made up of five working groups;

1. Terminology.
2. Biobanks and Bioresources.
3. Analytical methods.
4. Bioprocessing.
5. Data processing and integration.

The IMPROvED Biobank Manager Ms Emma Snapes participated in Working Group 2; Biobanks and Bioresources, and provided input to the drafting of this international standard. The aim of standardising mechanisms and introducing international benchmarks of excellence, is that research methodologies will become more rigorous, enhancing the quality of the research through participating in the Working Group, Ms Snapes was able to observe and interact with international experts in the field of biobanking to ensure that the IMPROvED biobank was not only future proofed for the introduction of the standard, but was adhering to current regulatory and international guidelines for biobanking standards including the International Society for Biological and Environmental Repositories (ISBER) Guidelines.

The ISBER is the largest international forum that addresses the technical, legal, ethical, and managerial issues relevant to repositories of biological and environmental specimens. It is a professional society of individuals and organizations who share an interest in promoting consistent, high quality standards, ethical principles and innovation in biospecimen banking, by uniting the global biobanking community. IMPROvED also attained membership of the European Society for Biopreservation and Biobanking (ESBB), a regional chapter of the ISBER. The ESBB is a society for people involved in the collection and storage of biological materials from all species. The society, focusses on Europe, the Middle East and Africa, and was founded in August 2010 with a mission to advance the field of biobanking in support of research relating to healthcare, agriculture and the environment.

MedSciNet proved to be a willing partner in the design, validation and implementation of a clinical database or electronic Case Report Form (eCRF), that, in additional to the capture of clinical data fields, also captured biospecimen quality information such as time, temperature, spin speed, etc. on an individual sample basis for each stage of sample storage and processing.

The IMPROvED Consortium has established a project and biospecimen quality management system to minimize pre-analytical variations. The pursuit of biobank quality remained a dynamic activity for the duration of the project, and continues to do so, given that the IMPROvED biobank is one of the greatest assets in the consortium.

1.3.5 IMPROvED Clinical Study of Screening Tests for Pre-eclampsia

All clinical trials are required to be conducted in accordance with the International Conference on Harmonisation-Good Clinical Practice (ICH-GCP) guidelines. ICH-GCP is an international ethical and scientific quality standard for the design, conduct, performance, monitoring, auditing, recording, analysis and reporting of clinical trials. The ICH E6 Guidance Document on Good Clinical Practice (ICH E6) provided a list of required documents for the conduct of a clinical trial. In compliance with these guidelines, Study Management and REGIONH established the Study Master File (SMF) and the Investigator Site File (ISF), for each recruitment site. The ISFs, which contain documentation for that specific recruitment centre, together constitute part of Study Master File (SMF), which was updated and maintained during the course of the study by UCC, the sponsor site. The SMF and ISFs are up to date and will be finalised following close out report competition and submission for individual ethics boards within the consortium. The Principal Investigators at each recruitment centre have now reviewed their respective ISF using a checklist for completeness provided by the IMPROvED Project Manager, to ensure that all required documentation has been acquired and filed appropriately.

All essential documents, as indicated by the checklists provided, are filed in the ISFs. The SMF remains at UCC. The IMPROvED Protocol states that essential study documents will be archived for at least 10 years after completion of the study, therefore all ISFs, signed informed consent forms and any pertinent source data will be archived at each recruitment centre for a minimum of this period of time.

1.3.6 Study Recruitment

IMPROvED aimed to recruit 5000 first time mothers in a phase IIa clinical study through seven recruitment centres based in five European countries. Actual recruitment took place in 5 recruitment centres (Figure 6). Recruiting in multiple centres and different countries ensured that participants were representative of different patient populations and healthcare models. Preparatory work for the clinical trial was completed in WP1 and WP2 (Figure 2). A European tender process resulted in unforeseen and unexpected delays in commencing recruitment. Recruitment took place in five recruiting sites, and recruitment progress was monitored throughout the study. Where a risk that recruitment targets would not be met was identified, pre-agreed contingency plans were activated. This included the consortium investigating which recruitment centres had the capacity to increase recruitment in a cost-efficient manner. Resources were reallocated, and recruitment targets altered accordingly to account for recruitment not taking place at all seven planned sites. The original scope of the study was expanded to include collecting additional biospecimen types at an additional number of time points. This significantly added to the quality of both the study and the biobank and will enhance the range of future research which can be accomplished using the IMPROvED residual biobank. It did, however, increase the work and staffing resources required per participant recruited.

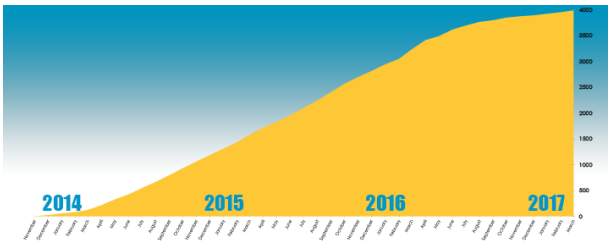


Figure 6: IMPROvED actual recruitment graph.

1.3.7 Clinical Dataset

Clinical and biobank data was entered to the IMPROvED database for each participant as she moved through the study from recruitment, visits, delivery and postnatal data. Comprehensive end of pregnancy outcome data were collected, entered to the IMPROvED database and subjected to monitoring by the local data monitors and the Global Clinical Coordinator who acted as the Global Data Monitor. Information on maternal medical history, delivery, the newborn infant, maternal postnatal data, and, if applicable, data recorded during admission to the Neonatal Unit was drawn from the participants' medical record and entered to the IMPROvED database. The IMPROvED database identified missing/incomplete compulsory data, and all data was locked by the local data monitor, prior to the local PI's final signature. MedSciNet performed data exports periodically for statistical analysis, and subsets of relevant variables will be sent to Metabolomic Diagnostics and MyCartis for inclusion in test algorithms. There are currently 899 variables recorded in the IMPROvED database. A Clinical Dataset Reports document deals with the data export required for algorithm testing and refinement, and statistical analysis.

1.3.8 Clinical Data Export

The selection of variables to take priority in data dictionary compilation are based on the 3 selection tasks; Variable Reduction/Outcome/Test Validation Integrity. MedSciNet provided two ways to export the final data set from the database – data queries and admin data export. The data queries allow the user to extract sets of data records according to a highly specialised specification inputted by the user. The system may be used to perform an in-depth analysis of data, or to identify records that meet a set of very specific criteria. The data export feature is available to Users who have been registered on the system as Global Administrators. This provided a convenient way to export all clinical data, together with the biobank data, to a Microsoft Excel spreadsheet. Work is ongoing within the consortium to get all data completed, monitored and signed off by the local Principal Investigators. Data entry will be completed in H1 2018. Once this is achieved, the first round of variable selection can begin.

1.3.9 Early Pregnancy Screening Test Development

1 in 20 first time pregnancies are complicated by pre-eclampsia, a leading cause of maternal and fetal morbidity and mortality. No clinically useful screening test exists; consequently, clinicians are unable to offer targeted surveillance or known/emerging preventative strategies. Consortium members had pioneered a personalised medicine approach to identify blood-borne biomarkers through technological advancements, particularly in the field of mass spectrometry and the comprehensive mapping of the blood metabolome and proteome.

The overall objective of the IMPROvED project was to develop a sensitive, specific, high-throughput and economically viable early pregnancy screening test for pre-eclampsia. This involved a multicentre, phase IIa clinical study to collect biosamples and clinical data to assess and refine novel and innovative prototype tests based on emerging metabolomic and proteomic technologies developed by the SMEs within the consortium, Metabolomic Diagnostics and MyCartis. The study aimed to

- Determine if prototype predictive assays and algorithms translate to the clinical environment.
- Assess if potential synergy of a combined metabolomic and proteomic approach.
- Progress regulatory approval and development of the selected test into the clinical arena.

The application of new technologies to identify ‘at risk’ patients in early pregnancy will allow stratified care with personalised fetal and maternal surveillance, early diagnosis and timely intervention. If an effective test could reduce antenatal visits by 50%, combined a 20% reduction in the incidence of disease through administration of therapies (such as aspirin) to those at risk, the potential savings would approximate to €4 billion of the estimated €9 billion/yr spent in Europe providing antenatal care for nulliparous women and treatment for pre-eclampsia. Moreover, an accurate predictive test would be a crucial step in reducing the life-threatening complications of the disease.

1.3.9.1 Proteomic Test Development

One of the main objectives of the IMPROvED project is to develop a clinically relevant pre-eclampsia risk stratification test applicable at mid gestation using protein biomarkers measured in blood.

The IMPROvED Consortium Partner SME MyCartis, previously traded under the name Pronota, which it changed in 2014 following the acquisition of the Swiss business unit of Biocartis. MyCartis planned to market its own multiplex platform, Evalution; an innovative multiplex technology designed for rapid and cost-effective measurement of a broad range of protein and nucleic acid biomarkers panels. Thus, the system was valuable for many clinical and pharmaceutical applications including the development of a pre-eclampsia risk stratification test.

Multiplex assays have a clear technological advantage to the more widely used singleplex ELISAs (as originally planned for the IMPROvED study). Multiplex assays, measure a panel of biomarkers is in a single test, reducing sampling and consumable costs, along with assay time. Furthermore, MyCartis’ proprietary microfluidic-based technology enables automated results, wide assay dynamic range, and high accuracy and precision, features which help produce high quality data.

Development of a multiplex pre-eclampsia panel involves several steps, such as reagent development; assay development (feasibility and optimization), and assay qualification. For each of the protein biomarkers of interest, proprietary antibody pairs and antigens were successfully developed and produced by recombinant technology. Proprietary produced materials were all quality checked (gel analysis, HPLC, absorbance measurement, etc.) before release and use by MyCartis for further experiments in assay development.

MyCartis engaged an experienced third party for the development of the multiplex pre-eclampsia panel and an exploratory study was performed in order to evaluate for each of the selected markers the performance of different antibody pairs (including both proprietary as well as commercially available materials). This exploratory study demonstrated that for each of the five selected markers, antibody pairs could be successfully selected on Evalution when tested in a singleplex format. Most of the proprietary reagents could be used to measure the biomarkers within or close to the expected concentration range in blood. This was the case for standard curves generated for sENG, PlGF, and SPINT. Singleplex assays for both MCAM and IGFALS showed to be too sensitive and that they would require further optimization.

In addition to evaluating the dynamic range of each of the markers, cross-reactivity was tested. No cross-reactivity was found for MCAM, ENG, or SPINT1. IGFALS showed, however, cross-reactivity with other capture antibodies (specifically PlGF capture antibody). This meant that blocking strategies and buffer optimizations would need to be further evaluated in order to resolve these issues. In addition, the stability of the reagents, which is crucial for a robust assay, would need to be assessed. Only once an assay is optimized, can it be qualified by assessing the analytical performance.

Based on the results from the exploratory study, multiplex assay development was initiated. A variety of assay conditions and analytical parameters were evaluated in order to reach the predefined assay specifications. Different criteria were analysed such as assay precision, sensitivity, linearity, parallelism, high dose hook effect, etc. In addition, the occurrence of interfering substances in plasma as well as the stability of the assay components were assessed. MyCartis choose final antibody pairs for IGFALS, MCAM, and sENG and worked towards finalising the selection for SPINT and the conditions for the other assay reagents (e.g. SA-PE and buffers).

Due to the broad variety in dynamic range between the markers of interest, where IGFALS is in the µg/ml range while the others are found in the pg to ng/ml range, and also the cross-reactivity found for IGFALS, it was decided to initially exclude IGFALS from the multiplex design and limit the development of this latter marker to a singleplex assay.

MyCartis also investigated sample preparation, especially for EDTA-plasma. This method solved the majority of the issues that were observed during the early phase of the development. However, the new method still failed to produce useful measurement for PlGF. Since MyCartis did not require PlGF measurement for their algorithm, it was decided to exclude it from the multiplex panel which would thus be consisting of MCAM, sENG, and SPINT.

It was planned that the performance of the near-finished assay would be assessed for sensitivity, precision, accuracy, and robustness. This would allow MyCartis to identify remaining areas for optimisation before locking the assay design. Once an assay design is locked, the next step is to enter assay validation phase. A series of experiments (derived from CLSI guidelines) were planned to be performed. The resulting data would then be used for assessing the analytical performance of the assay. If the assay performance meets the target specification, MyCartis would then enter SCOPE sample testing phase in which all available clinical samples from the SCOPE consortium will be analysed, which will allow MyCartis to fine-tune and lock the assay algorithm. In preparation for this, Material Transfer Agreements with the SCOPE centres from Cork, Manchester, Leeds, and London were finalised, and the majority of required SCOPE clinical samples were transferred to MyCartis.

In September 2017, MyCartis concluded that continued efforts towards fulfilment of the developmental work would not be pursued. This decision was based on following factors:

1. The multiplex assay development against targets M-CAM/SPINT1/ sENG/IGFAL faced severe technical issues at 2 points:

(i) The IGFAL detection antibody cross reacted with capture antibodies against M-CAM, SPINT1, and sENG, which results in false analytical positive results.

(ii) Inadequate analytical sensitivity for SPINT1. MyCartis screened the performance of commercially available antibodies and their proprietary antibodies, but none of the combinations could detect SPINT1 in the relevant concentration range (<10 ng/mL) found in the samples.

2. Mitigation strategies, through extending elaborate assay development efforts, were defined and pursued. This yielded the following primary results:

(i)The necessary SPINT1 sensitivity could be achieved by multivariate screening of additional assay reagents and parameters. However, inclusion into a multiplex format is not possible due to a different sample dilution factor required under these new conditions, which conflicts with the analytical range that was established for the other targets in the biomarker panel.

(ii) IGFAL cannot be incorporated into the multiplex assay despite extensive further assay optimization efforts to resolve antibody cross-reactivity. It would require *de novo* antibody development. At that time, MyCartis assessed that even if *de novo* antibody discovery and development was pursued, there was a significant residual risk that this might not result in the required analytical performance of the IGFAL assay in the biomarker panel.

Detailed planning revealed that it was impossible to complete the remaining work within the foreseen timeframe and that the intensive assay redevelopment efforts had taken up large parts of the remaining budget and time. Additionally, clinical practice guidelines for screening of pre-eclampsia had changed since the IMPROvED project inception and an assessment of current clinical practice in the screening for pre-term pre-eclampsia risk demonstrated that diagnosis/prognosis of pre-eclampsia risk is increasingly performed at earlier time-points in the pregnancy with the ASPRE study demonstrating the effects of aspirin use following screening at 11-13 weeks' gestation.[3] [4] The business context was re-evaluated and it was concluded that the commercial potential for the protein-based multiplexing assay under development would not, and did not, justify the potential future investments to be made particularly in light of the development issues MyCartis were facing.

No additional activities were planned by MyCartis, the IMPROvED WP4 Leader MyCartis performed the formal close-out by reporting and arranging the shipment of biological samples were sent back to UCC biorepository.

1.3.9.2 Metabolomic Test Development

Within the framework of the IMPROvED project, Metabolomic Diagnostics Limited (METABOL) has utilised its knowledge and experience in the area of Mass Spectrometry (MS), Regulatory Affairs and In Vitro Diagnostics (IVD) design to progress the development of product prototypes that will form the basis for a pre-eclampsia risk stratification predictive screening test to screen first time pregnant women at approximately 15 weeks of gestation.

Currently METABOL is one of the few Small to Medium Enterprises (SME) in Europe which is ISO:13485 certified for the design, development of IVD devices. Certification was achieved in 03/2015, and this certification was successfully maintained ever since. Being compliant with internationally recognised standards relevant to Design and Manufacturing of IVD will expedite the time of bringing MetTest to the market. The same standard is applicable to manufacturing. METABOL will seek to expand its ISO:13485 accreditations to include manufacturing in advance of MetTest being ready for validation in IMPROvED.

"MetTest" development efforts performed by METABOL during IMPROvED

At the start of IMPROvED METABOL had a selection of metabolite biomarkers available, which were shown to be relevant to the prognosis of pre-eclampsia. In order to translate this list of metabolites into an IVD product, METABOL embarked on the development of a clinical assay based on the application of liquid chromatography hyphenated with mass spectrometry (LCMS). This technology allows for the simultaneous and highly specific analysis of multiple biomarkers.

This ability to multiplex many analyses is currently being recognised within the clinical laboratory market as a highly desirable attribute in view of the move toward more holistic, personalized diagnostic solutions. As a result, we are currently witnessing a paradigm shift in the clinical laboratory market, whereby mass spectrometry is increasingly replacing classic IVD platforms, with METABOL being one of the very few companies worldwide developing novel clinical tests directly on mass spectrometry platforms (different from companies migrating existing tests from older technologies to mass spectrometry), METABOL established itself, during the IMPROvED project, as a leader in this IVD innovation space.

To deliver upon its goal of developing a pre-eclampsia risk prediction test ("MetTest") for clinical validation in the IMPROvED clinical study, METABOL set out to firstly develop prototype assays for all the metabolite biomarkers of interest and then to perform a research verification studies of MetTest.

A strategy of continued improvement, involving an iterative process of verifying the developed MetTest assays were 'fit-for-purpose' and exploring whether the metabolite quantifications enabled the development of performant prognostic algorithms. To enable execution of this task, a research collaboration agreement was established between the company and the international SCreening fOr Pregnancy Endpoints (SCOPE) consortium as per the IMPROvED DoW, enabling METABOL to verify its MetTest iterative development progress by executing case:control studies. It was anticipated that an iterative process would enable the step wise optimization of both the MetTest technology and the risk stratification algorithm, as schematised below (Figure 7).

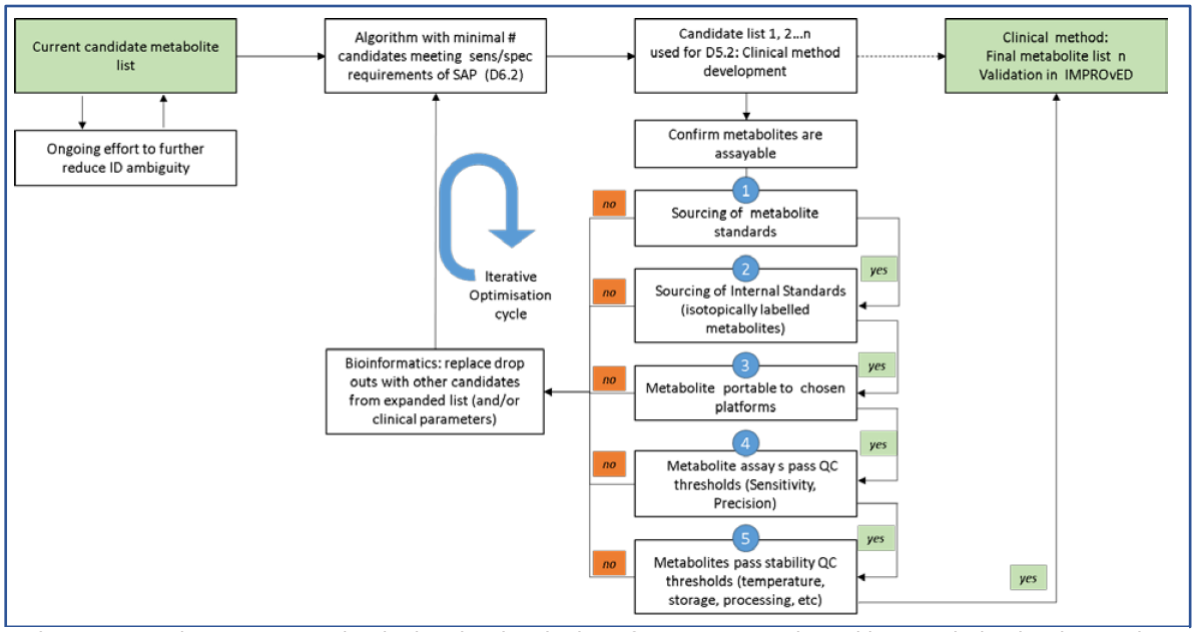


Figure 7: Iterative process underpinning the developing of MetTest, as adopted by Metabolomic Diagnostics from the start of IMPROvED.

However, METABOL soon found that a) not all of the original metabolite markers were amenable to LCMS, and at a later stage that b) its initial multiplex assay prototypes were not fit for purpose. At that time, METABOL engaged in the execution of the Contingencies and Corrective Actions plan resulting in the bottom-up re-engineering of all key components of the MetTest platform.

In Q1 2017 METABOL delivered a robust metabolomics translational platform, "MetaDxSCOUT",considered to be an (internal) product in its own right. In brief, the analytical components of MetaDxSCOUT constitute (see also Figure 8):

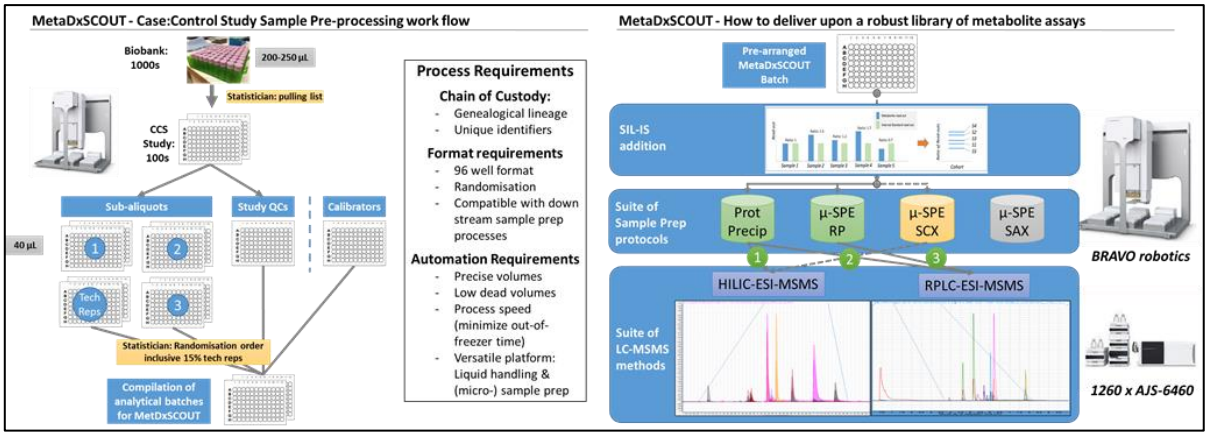


Figure 8: Schematic representation of the analytic components of MetaDxSCOUT.

1. A suite of study design and quality assurance bioinformatic tools.

2. A robotics assisted sample preparation protocol, which processes 96 samples at a time. Implementation of a high degree of automation in sample work-up process delivered well-controlled pre-analytical variability, which in turn has a positive impact on the overall precision.

3. The implementation of 2 separate LC-MS methods to analyse the worked-up samples.

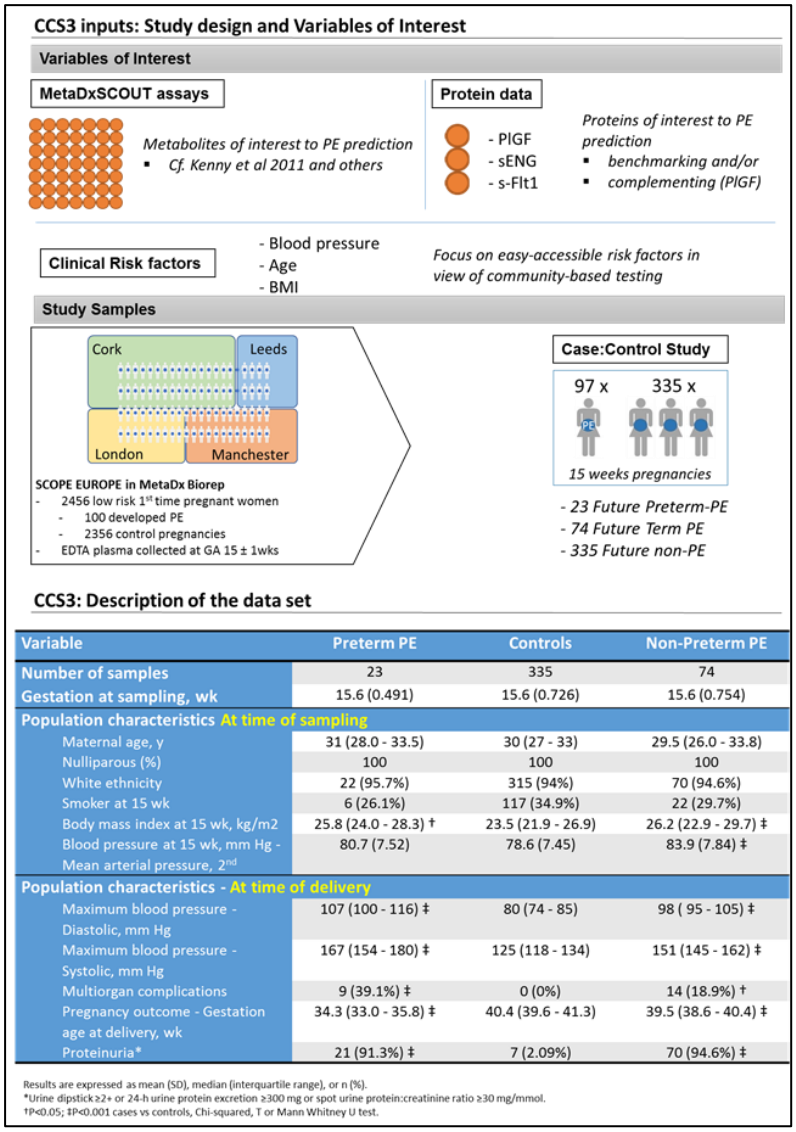
    a. The 2 chromatographic are complementary with regards to their ability to resolve specific sub-sets of target metabolites of interest.

    b. The use of 2 LC-MS also allowed for more optimal parametrisation of the mass spectrometric detection methods.

    c. The comprehensive implementation of Stable Isotope Labelled Internal Standards, in line with best practices,[5] to support precise quantification and unambiguous identification of the target metabolites (analytical specificity).

With MetaDxSCOUT, METABOL developed a proprietary translational research pipeline capable of generating the high quality datasets required to verify the metabolite constituents of "MetTest".

Upon delivery of MetaDxSCOUT, METABOL executed a final case:control study within the SCOPE collaborative framework, i.e., CCS3.[6 7] During this study more than 50 metabolites of interest were assayed, and following Quality Control, 46 metabolite quantifications were retained for algorithm development.



CCS3: Description of the data set

Variable	Preterm PE	Controls	Non-Preterm PE
Number of samples	23	335	74
Gestation at sampling, wk	15.6 (0.491)	15.6 (0.726)	15.6 (0.754)
Population characteristics At time of sampling			
Maternal age, y	31 (28.0 - 33.5)	30 (27 - 33)	29.5 (26.0 - 33.8)
Nulliparous (%)	100	100	100
White ethnicity	22 (95.7%)	315 (94%)	70 (94.6%)
Smoker at 15 wk	6 (26.1%)	117 (34.9%)	22 (29.7%)
Body mass index at 15 wk, kg/m2	25.8 (24.0 - 28.3) †	23.5 (21.9 - 26.9)	26.2 (22.9 - 29.7) ‡
Blood pressure at 15 wk, mm Hg - Mean arterial pressure, 2nd	80.7 (7.52)	78.6 (7.45)	83.9 (7.84) ‡
Population characteristics - At time of delivery			
Maximum blood pressure - Diastolic, mm Hg	107 (100 - 116) ‡	80 (74 - 85)	98 (95 - 105) ‡
Maximum blood pressure - Systolic, mm Hg	167 (154 - 180) ‡	125 (118 - 134)	151 (145 - 162) ‡
Multiorgan complications	9 (39.1%) ‡	0 (0%)	14 (18.9%) †
Pregnancy outcome - Gestation age at delivery, wk	34.3 (33.0 - 35.8) ‡	40.4 (39.6 - 41.3)	39.5 (38.6 - 40.4) ‡
Proteinuria*	21 (91.3%) ‡	7 (2.09%)	70 (94.6%) ‡

Results are expressed as mean (SD), median (interquartile range), or n (%).
*Urine dipstick ≥2+ or 24-h urine protein excretion ≥300 mg or spot urine protein:creatinine ratio ≥30 mg/mmol.
†P<0.05; ‡P<0.001 cases vs controls, Chi-squared, T or Mann Whitney U test.

Figure 9: Summary CCS3 study. CCS3 underpins D5.4 - Selection of MetTest Algorithm

In Figure 9, the CCS3 study design, the description of the patient cohort and the variables considered inputs and design are summarised; the CCS3 study design was aligned with the (refined) IMPROvED goals as further elaborated later.

As mentioned earlier, METABOL adopted an iterative process of alternating product development steps with case:control studies for homing the final product requirement specifications of MetTest in advance of MetTest's (technical) verification and (clinical) validation in IMPROvED. In this regard MetaDxSCOUT, METABOL's proprietary translational metabolomics platform, is a direct consequence of this iterative strategy.

At the same time, when it was realised early in the IMPROvED project, that it was impossible to develop robust assays for all of the 14 metabolites underpinning the initial version of MetTest,[7] METABOL took the opportunity to develop novel strategies to identify novel pre-eclampsia risk stratification algorithms, leveraging contemporary insights in the pathophysiological origins of pre-eclampsia as emerging during IMPROvED.

Development of an alternative data analysis strategy to identify MetTest Algorithm

Underlying these novel prototype algorithms is the concept that pre-eclampsia is not a single disease, but a syndrome.[8] This fundamental understanding that pre-eclampsia is not a single disease entity has been at the heart of METABOL's de novo algorithm development efforts. In brief, it is based on two pillars:

1) To encapsulate the complexity of a multifactorial syndrome, measurement of multiple biomarkers is required.

2) To ensure that each biomarker's potential is fully "unveiled" it is key that the biomarker panels are aligned with the right populations.

For MetTest to have clinical utility, it is important that – apart from being rooted in pathophysiological insights – the delineation of sub-populations or/and outcome subtypes in proxy phenotypes is simple and well aligned with clinical practice. This resulted in the following delineations being investigated (Figure 10):

1. Prediction of Pre-eclampsia in all nulliparous (no delineation applied).
2. Delineation in function of gestational age at delivery, i.e.,
    a. prediction of Preterm pre-eclampsia, i.e., pre-eclampsia that warrants for delivery of the infant before 37 weeks of gestation.
    b. prediction of Term pre-eclampsia, i.e., pre-eclampsia that warrants for delivery of the infant after 37 weeks of gestation.

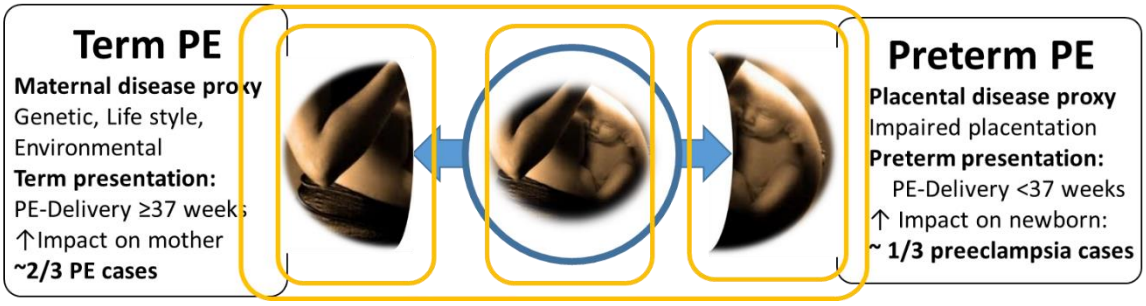


Figure 10: Delineation of the Pre-eclampsia Prediction challenge.

Establishing Performance Targets for MetTest_All pre-eclampsia

With the participant recruitment into IMPROvED extended beyond the initial timeline, METABOL has taken the opportunity to re-inform itself on the optimal MetTest performance thresholds. With demands of obstetricians, pregnant women and health care systems changing over time, METABOL has performed additional market research and KOL consultation to confirm the performance requirements for MetTest.

In view of the original IMPROvED goal for MetTest. i.e., the prediction of All-Pre-eclampsia in low risk nulliparous, METABOL consulted with several KOL worldwide, as well as with clinicians of the IMPROvED consortium on establishing performance targets for MetTest.

To inform the performance targets for MetTest, the prenatal care of multiparous women in function of their pre-eclampsia risk was evaluated.

The prenatal management of a multiparous woman with regards to pre-eclampsia is largely guided by her previous pregnancy history. Epidemiological studies have shown that previous pre-eclampsia is associated with an increased risk of recurrence. For a second pregnancy, recurrence risks of about 1 in 6.8 to 1 in 8.6 (or Positive Predictive Value (PPV) of 0.116 to 0.147) are reported,[9 10] whereas a woman without prior pre-eclampsia, will have a lower risk of 1 in 77 to 1 in 100 (or Negative Predictive Value (NPV) of 0.987 to 0.99).[9 10] In line with this, if a woman has experienced

pre-eclampsia in a previous pregnancy, she will be managed more vigilantly in most healthcare systems in high resource settings, with more prenatal visits compared to a woman who did not develop pre-eclampsia in any earlier pregnancy.

Based on the above, we proposed that a pre-eclampsia risk stratification test for nulliparous should ideally mimic the pre-eclampsia risk information as available for a second-time pregnant woman. Therefore, the test should either stratify nulliparous women to a high-risk group with a post-test pre-eclampsia probability of at least 1 in 7.5 (equivalent to a PPV = 0.133; rule-in) or stratify them to a low-risk group with a post-test probability of at least 1 in 90 (equivalent to a NPV = 0.988; rule-out) and ideally both (Figure 11).

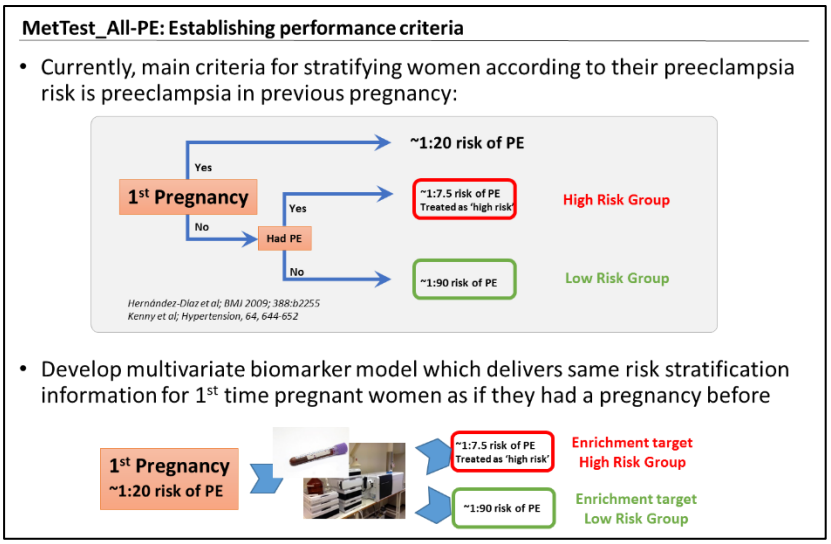


Figure 11 Performance targets for MetTest_All pre-eclampsia as inferred from the pre-eclampsia risk in multiparous women with and without previous pre-eclampsia.

Interestingly, the statistical tool of choice to evaluate the performance of diagnostic / prognostic tests, i.e., the Receiver Operating Characteristic (ROC), is ill-suited when it comes to using PPV- or/and NPV targets as target thresholds. At the same time, predictive values are most relevant in assessing the relevance of a prognostic test as they relate more directly with clinical utility. To overcome the limitations of the well-established ROC framework to assess prognostic tests, METABOL scientists, and collaborators, established a novel statistical methodology which allows for the direct use of Predictive Value thresholds in the evaluation of novel prognostic tests; this methodology was published in 11/2017.[11]

In Figure 12, this methodology was used to plot both the proposed minimal PPV (0.133) and NPV (0.988) criteria on the ROC space to identify the quadrant in the ROC space which would comply with both these criteria simultaneously. To illustrate the impact of prevalence, the criteria for three published prevalence values were plotted: 0.05,[7] 0.03,[12] and 0.07[13] (rounded for convenience).

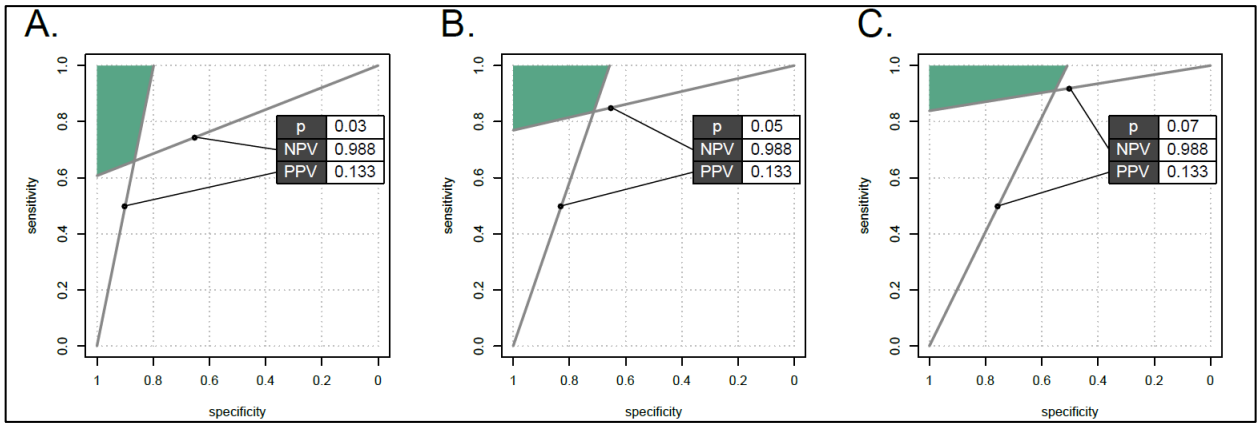


Figure 12: Equi-PPV and equi-NPV lines corresponding the pre-eclampsia risk in multiparous pregnant women with previous pre-eclampsia (PPV) or without previous pre-eclampsia (NPV). These minimal prognostic performance thresholds are calculated for three different pre-eclampsia prevalence values, as reported for first time pregnant women.

It can be appreciated that to achieve the success quadrant in each of the possible prevalence scenarios, a screening test with extraordinary Sensitivity and Specificity is required. The existence of such a test is unlikely; for instance, Royston et al. noted that the AUROC of prognostic models is typically between 0.6 and 0.85.[14] Knowing that pre-eclampsia is a syndrome,[8] that at time of risk prediction the future disease status remains to be determined by a stochastic process, the target population concerns healthy first time pregnant women without any overt risk factors, and pre-eclampsia diagnoses can't be made unequivocally, the absence of such a test should not be surprising.

Upon the realisation a single prognostic test for pre-eclampsia in low risk first time pregnant women it was unlikely to meet the earlier proposed target PPV and NPV criterions simultaneously, we hypothesise that possibly more meaningful pre-eclampsia risk prediction can be achieved when the risk stratification question is resolved in its two constituting requirements: i.e., treat the rule-in and rule-out independently. Instead of a pursuing a single risk stratification test which meets both clinical PPV and NPV requisites, the development of separate rule-out and rule-in tests which complement each other and which can be deployed together, should be considered (Figure 13).

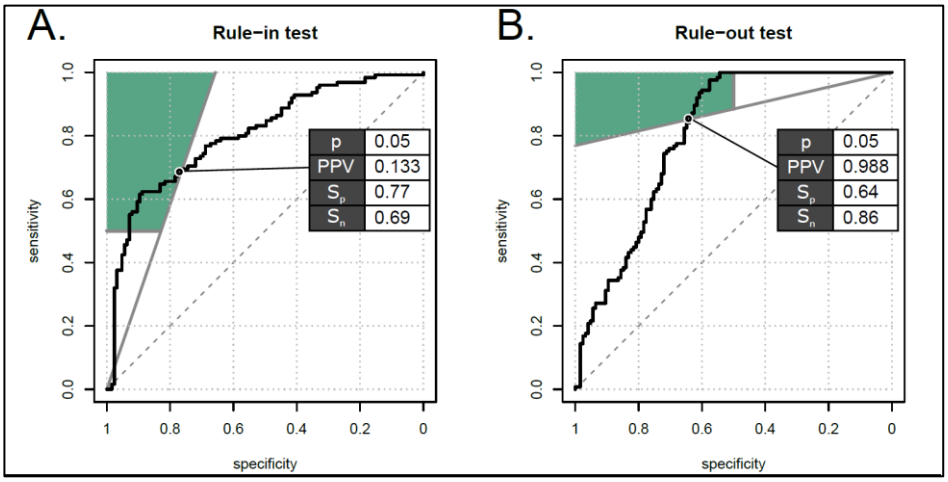


*Figure 13: Multivariable modelling. Two possible prognostic test permutations for pre-eclampsia prediction in first time pregnant women are shown (hypothetical data) **A.** Rule-in test compliant with pre-set test specification: PPV ≥ 0.133, Sn ≥ 0.50, for a 5% disease prevalence. **B.** Rule-out test compliant with pre-set test specification: NPV ≥ 0.988, Sp ≥ 0.50, for a 5% disease prevalence.*

Establishing Performance Targets for MetTest_PTPE (or PrePsia™)

Since the start of IMPROvED, the use of low dose aspirin (LDA) as a prophylactic treatment for the prevention of preterm pre-eclampsia cases has gained momentum following the publication of the meta-analysis of Bujold et al.[15] In this report, it was inferred that prevention of pre-eclampsia by prophylactic treatment with LDA required starting the treatment before 16 weeks of gestation. In 2017, this finding was validated in the ASPRE study,[3 4] a reduction of 62% in Preterm pre-eclampsia incidence was found upon treating pregnant women at risk of preterm pre-eclampsia with LDA.

MetTest can support a similar risk stratification – LDA treatment paradigm based on timely LDA administration as MetTest is applied at ca. 15 weeks of gestation; and possibly earlier – cf. the first trimester specimens as collected under IMPROvED. **The alignment of MetTest with a (low risk) drug intervention protocol is very positive both from a commercial as well as a clinical utility point of view.**

Within the ASPRE study, a Preterm pre-eclampsia risk algorithm was used as developed by Nicolaides et. al.[3 4] Whereas this algorithm delivered good detection rate for Preterm pre-eclampsia of about 77% at a screen positive rate of 10.5%,[3] it is using a number of inputs which are either not relevant to low risk nulliparous or/and not trivial (and expensive) to deploy in a primary care screening setting. A development goal for MetTest was therefore set at achieving similar detection rates for future preterm Pre-eclampsia (rule-in) cases and pregnancies that will not be affected preterm pre-eclampsia later in their pregnancies. For reference, an overview of the variables applicable to the algorithm as used in the ASPRE study are tabulated in Figure 14.

	Algorithm Variables	ASPRE	Relevant to IMPROvED population	Comment
Patient Characteristics	*Ethnicity*	✔	✔	
	Smoking status	✔	✔	
	Chronic hypertension	✔	NO	strong predictor
Pregnancy History	*Parity*	✔	NO	IMPROvED: all Nullips
	Preeclampsia History	✔	NO	strong predictor
Clinical Measurements	*Weight & Length*	✔	✔	
	BMI	✔	✔	
	Mean Arterial Pressure		✔	
Biomarkers		*2*	*TBD*	
Ultrasound	*Sonographer certification(FMF)*	✔	NO	*restricts utility for primary care setting / expensive / Single certification body impacts roll-out*
	Uterine Artery Doppler	✔	NO	*restricts utility for primary care setting / expensive*
Detection Rate			77.00%	

Figure 14: Variables used in the Preterm Pre-eclampsia predictor within the ASPRE trial. Assessment of the relevance of the variables with regards to 1) prediction in nulliparous without overt risk factors and 2) widespread adoption in a primary care screening context.

During the IMPROvED period, there were significant developments in the pre-eclampsia prognosis/diagnosis market space. Most notably, PlGF became accepted as a marker with clinical utility to stratify women presenting with suspected, but unconfirmed pre-eclampsia, according to their risk of experiencing an adverse pregnancy outcome in 1 to 2 weeks following clinical presentation.[16] Whereas this diagnostic application of PlGF is different from the prediction (or prognosis) of pre-eclampsia, it's acceptance in the market place resulted in PlGF being integrated in the test portfolios of the major IVD companies worldwide. Consequential to this, there is an interest in utilising PlGF also in (preterm) pre-eclampsia risk prediction.

However, the predictive performance of PlGF as a stand-alone prognostic biomarker is limited; for instance, in CCS3, PlGF will deliver ca. 40% detection rate at PPV = 0.07. The earlier mentioned preterm pre-eclampsia predictor as developed by Nicolaides et al. overcomes the limited prognostic ability of PlGF by supplementing it with a plethora of other variables (cf. Figure 14), yet many of these variables are not available in 1st time pregnant women, the pregnancy population within IMPROvED.

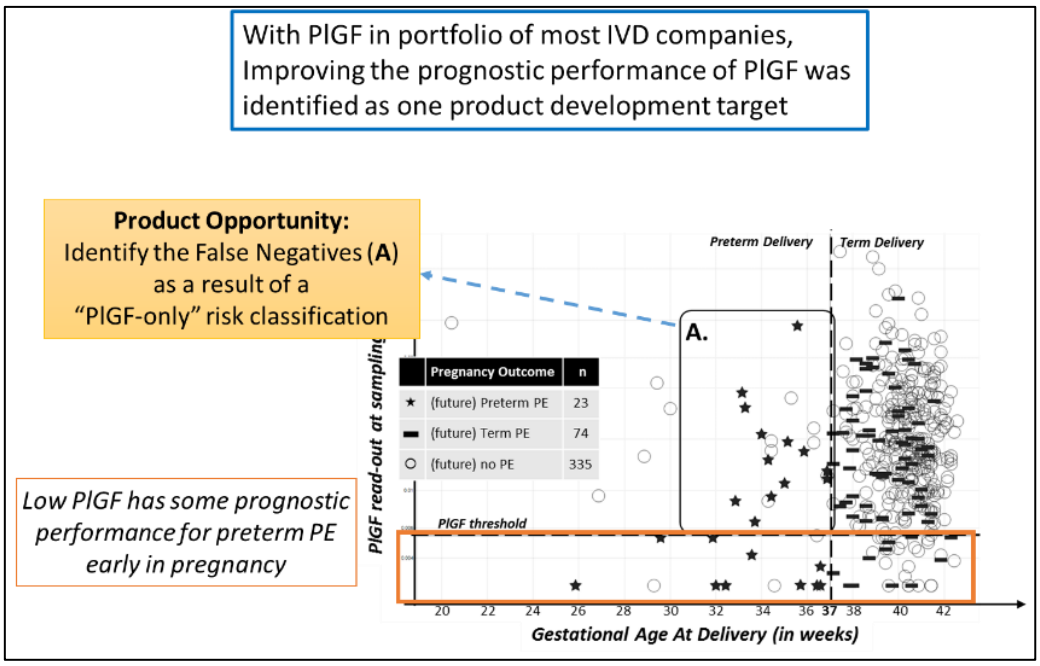


Figure *15: The strengths and limitations of PlGF with regards to risk stratifying pregnancies according to their future risk of developing Preterm Pre-eclampsia (exemplified with actual data – CCS3).*

With PlGF being established in the market, and PlGF having distinct, albeit limited, prognostic performance METABOL specifically investigated whether its portfolio of metabolites could complement PlGF.

Figure 15 summarizes the limitations of PlGF and thus the MetTest_PTPE product opportunity, i.e., resolving the False Negative results following the application of PlGF-based stratification.

Within CCS3, METABOL found a set of algorithms with exceptional classification potential. These classifiers simultaneously meet the pre-defined rule-in and rule-out criteria. With these classifiers, the triaging of nulliparous women in either a high-risk group, i.e., a woman has a risk >= 1/14 to develop preterm pre-eclampsia (or PPV >= 0.07), or a low-risk group, i.e., a woman has a risk =<1/400 to develop preterm Pre-eclampsia (or NPV >= 0.9975).

In view of the favourable results found for the prediction of Preterm Pre-eclampsia and the current market environment, METABOL has prioritised MetTest_PTPE classifiers to become METABOL'S first product, **PrePsia™.** The markers underpinning PrePsia™ will therefore be amongst the key metabolites that will be progressed to technical and clinical validation within the IMPROvED framework.

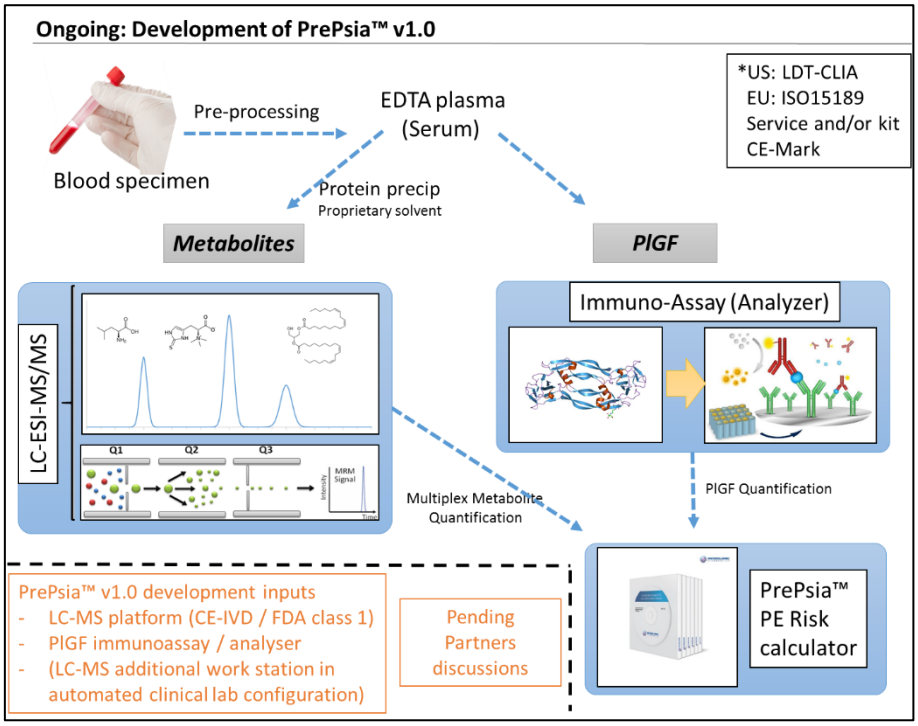
Moreover, compared to the earlier mentioned Preterm Pre-eclampsia stratification algorithm, PrePsia™ shows potential to deliver better classification, solely based on biomarker read-outs. If this prognostic performance can be validated within IMPROvED, PrePsia™ will establish itself as the better product in the market, following its ability to also stratify nulliparous women accurately and its intrinsic compatibility with a primary care screening setting (Figure 16).

	Algorithm Variables	ASPRE	PrePsia™
Patient Characteristics	*Ethnicity*	✔	
	Smoking status	✔	
	Chronic hypertension	✔	
Pregnancy History	*Parity*	✔	
	Preeclampsia History	✔	
Clinical Measurements	*Weight & Length*	✔	
	BMI	✔	
	Mean Arterial Pressure	✔	
Biomarkers		*PlGF, PAPPA*	*PlGF + 3 Metabolites*
Ultrasound	*Sonographer certification*	✔	
	Uterine Artery Doppler	✔	
Detection Rate		77%	91%

Figure 16 Comparison of the variables used in the Preterm Pre-eclampsia predictor within the ASPRE trial and PrePsia™, the latter will be subjected to technical and clinical validation in IMPROvED

Selection of the optimum format to deliver PrePsia™ to the market

With PrePsia™, METABOL's first version of MetTest, to be progressed to full productization, and sub-sequent technical and clinical validation within the IMPROvED framework, the optimum and final analytical platform will be a hybrid. A multiplex LC-MS assay will be used for the simultaneous analysis of the metabolites of interest whereas the PlGF analysis will be done using a suitable classic clinical analyser (Figure 17). METABOL is currently engaging with IVD providers to select the PlGF assay of choice to be used in the clinical validation. In parallel, METABOL has started the final development of the LC-MSMS component of PrePsia™.



2

Figure17 Schematic representation of the PrePsia™ test in preparation by METABOL in view of PrePsia™ technical and Clinical Validation in IMPROvED.

At the end of the formal IMPROvED project timeline METABOL did not progress to the analysis of the IMPROvED cohort, as a result of the following:

1. A delay in recruitment of the clinical cohort.
2. An operational delay in transfer of IMPROvED specimens from the central biorepository at UCC to METABOL.
3. The initial setbacks in terms of development of the enabling technology (MetaDxSCOUT) and the selection of the risk prediction algorithm for validation (see higher). This in turn impacted on the final product development of MetTest.
4. The opportunity for the consolidation the novel IP which arose from the corrective actions applied. This prevented the timely provision of inputs to REGIONH (The Copenhagen Trial Unit) regarding the IMPROvED Statistical Analysis Plan.

Yet, at time of reporting, the key reasons for delay are being resolved.

Next Steps: Technical and Clinical Validation of MetTest

From early in the IMPROvED project, METABOL has been careful to put in place the appropriate framework to support the technical and clinical validation of MetTest (PrePsia™).

Because MetTest is classified as an IVD (which is a Medical Device in the EU and US), METABOL started implementing ISO:13485, the recognised international quality standard governing quality management systems for medical devices from the start of IMPROvED. METABOL submitted itself to an audit to ISO:13485 by an independent management systems certification body in 2015. METABOL successfully passed this audit **and was certified to ISO: 13485, with scope covering "Development and Design of In Vitro Diagnostic Products"**. Since then METABOL has maintained its certification, and successfully passed its ISO: 13485 renewal audit in February 2018. Herewith, METABOL demonstrates the commitment and qualification to conduct its MetTest product development in agreement with international quality standards.

Because the preterm pre-eclampsia prognostic test, i.e., PrePsia™, is intended to be marketed in both the EU and US, it was decided early on in MetTest (PrePsia™) development process that all technical components with a potential impact on the quality of the finished product should be tested to a patient health level of quality assurance. This is in line with FDA and EU requirements to adopt a risk-based approach from a patient safety perspective to validation. In line with this requirement, METABOL will continue to perform the appropriate risk assessments in accordance with ISO:14971.

With a quality management system in place that meets the requirements of both US FDA 21 CFR 820 and EU IVDD Medical Device Directive, and the recent IVDR (REGULATION (EU), METABOL has a structure in place to collate and govern the necessary documentary evidence of the complete product development process for MetTest / PrePsia™.

In view of getting PrePsia™ ready for clinical validation in IMPROvED, METABOL has now started defining the requirements for the technical documentation required to demonstrate compliance of PrePsia™ with the Essential Principles of Safety and Performance of Medical Devices, as laid out in ANNEX I of the recently published IVDR 2017/746).

At the same time METABOL is consulting with various stakeholders regarding the desirable technical specifications for PrePsia™, for example, in terms of test Turn Around Times, and quantification regimens. These in turn will have an impact on the technical verification and technical validation plans.

Building on success

The significant advancements made by METABOL in the development of a truly novel clinical test, with the potential to impact on prenatal care globally, enabled METABOL to successfully raise

several rounds of capital (Venture and Private) during the IMPROvED period. This funding was used to complement the EU funding received under IMPROvED to fuel development of MetaDxSCOUT and METABOLs ISO13485 compliant infrastructure. This private capital ensures METABOL will finalise the MetTest development and validation work as set-out in IMPROvED, and as such honour its commitments to the pregnant women across Europe, its clinical collaborators in IMPROvED, and the European Commission.

Moreover, building on the significant progress made during the IMPROvED project, METABOL established an ambitious business plan to introduce PrePsia™ into the European market and beyond. In recognition of the disruptive potential of Metabolomic Diagnostics' and its first product PrePsia™, the company has been awarded €2 million in EU Funding under the latest Horizon 2020 SME Instrument Horizon 2020 call in March 2018.

2.1.1.1 Mathematical Assessment and Modelling of Potential Synergies of the Tests

Pre-eclampsia is a leading cause of maternal and infant morbidity and mortality. There are no clinically useful screening tests and clinicians are unable to offer targeted surveillance or preventative strategies. The IMPROvED project aimed to develop high-risk stratifications for nulliparous singleton pregnancies with different care pathways, to be agreed by different processes, which could be applied between weeks 14 and 16 of pregnancy. This would help clinicians identify groups of pregnant women for whom who preventive treatment and follow-up will be cost beneficial. The combined data and biobank collected during the IMPROvED study is useful as a tool to search for relevant high-risk groups within the framework of a controlled clinical trial.

The present statistical analysis plan included the development and validation of a prediction model of term and preterm pre-eclampsia based on clinical predictors. This model will be augmented by four novel biomarkers. The plan for this latter analysis was described in the Statistical Analysis Plan, although its execution has had to be postponed until the development of the biomarker assays.

2.1.1.2 Statistical Analysis

A statistical analysis plan is in preparation and feasibility tests have been conducted to check whether data transfer from data providers (MEDSCINET (clinical data); Metabolomic Diagnostics (diagnostic and prognostic test data)) to the IMPROvED statistical centre (REGIONH at The Copenhagen Trial Unit) is feasible, reliable, and 100%, reciprocally compliant. Work with these tests is not yet completed, as the final cleaned data from MEDSCINET and diagnostic and prognostic test data from Metabolomic Diagnostics were not transferred to REGIONH due to delays in recruitment. The data from MEDSCINET is expected during June 2018, and the data from Metabolomic Diagnostics is expected in November 2018. Hence, the final statistical analyses have been postponed to late 2018 or early 2019. The final detailed statistical report including all validation results will be submitted after conducting the statistical analyses on the diagnostic and prognostic tests.

2.1.1.3 Health Economic Assessment

This health economic report covered the early cost-effectiveness analysis of the pre-eclampsia screening test. A health economic model (decision tree) was developed using information from published literature, an online survey in healthcare providers, and the IMPROvED cohort. The model assumed that women, identified by the test to be at high risk, would receive intensified monitoring and prophylactic aspirin, for which the effectiveness was varied based on different sources of evidence (base case and best case). It was modelled that women classified as at low risk to develop pre-eclampsia, would need less visits and check-ups than current standard care. In the absence of final and validated test accuracy data (i.e. awaiting final validation of the test in the IMPROvED cohort), the model simulated 25 plausible scenarios in which the sensitivity and specificity of the test were varied, in order to set a benchmark for the minimum test performance that is needed for the test to become cost-effective. The main outcome was incremental costs per pre-eclampsia case averted, expressed as an incremental cost-effectiveness ratio (ICER).

Deterministic and probabilistic sensitivity analyses were conducted to assess uncertainty. Base case results, using a conservative efficacy estimate for prophylactic aspirin, showed that screening for pre-eclampsia would be a dominant option compared to the current situation in the UK. In the Netherlands, the majority of scenarios would be cost-effective from a threshold of €50,000 per pre-eclampsia case averted, while in Ireland and Sweden, the vast majority of scenarios would be considered cost-effective only when a threshold of €100,000 was used. In the best-case analyses, incorporating a more optimistic estimate of aspirin efficacy, ICERs were more favourable in all four participating countries. The screening strategy remained dominant in the UK, while in the Netherlands and Ireland, the test would be cost-effective at a lower threshold of €10,000 per pre-eclampsia case averted. In Sweden, the screening test would be cost-effective at a threshold of €30,000 or higher. Aspirin effectiveness, prevalence of pre-eclampsia, accuracy of the screening test and cost of regular antenatal care were identified as driving factors for the cost-effectiveness of screening for pre-eclampsia. In conclusion, screening for pre-eclampsia is potentially cost-effective. The model can be used to insert final test characteristics once they become available.

In conclusion, in this assessment of cost-effectiveness of early screening for pre-eclampsia, we have shown that there were some general important parameters that drive the cost-effectiveness. Further economic evaluation studies and long-term follow up based on proven accuracy of the test that take into account these parameters will be required to evaluate if the new screening test for pre-eclampsia can be a cost-effective option compared to the current situation.

2.1.1.4 Feasibility

Evaluation of the (sequential) application of MetTest (+/- 15 weeks) and Protest (+/- 20 week) had been proposed, in order to assess whether predictive performance could be significantly improved by combining the two tests under evaluation in IMPROvED. In this situation, an expansive feasibility study, investigating the technical, regulatory, and commercial aspects of such combination was to be conducted. However, due to technical constraints in the late-development stage and the perceived lack of clinical benefits of the 20 weeks ProTest protein-based multimarker panel due to new and emerging clinical treatment guidelines MyCartis choose not to pursue the development of their Protest.

Biomarker panels based on metabolites alone have to date failed to achieve the necessary predictive accuracy at 15 weeks of pregnancy. However, against the background of Pronota/Mycartis leaving the consortium, Metabolomic Diagnostics established that a combination of known proteins and metabolites had promising predictive potential for preterm pre-eclampsia (and possibly beyond preterm pre-eclampsia). The latter prototype test had been referred to as MetTest PTPE or PrePsia™, its proposed product name. Hence, some aspects of the technical feasibility of combining two types of biomolecules, remained relevant to PrePsia™.

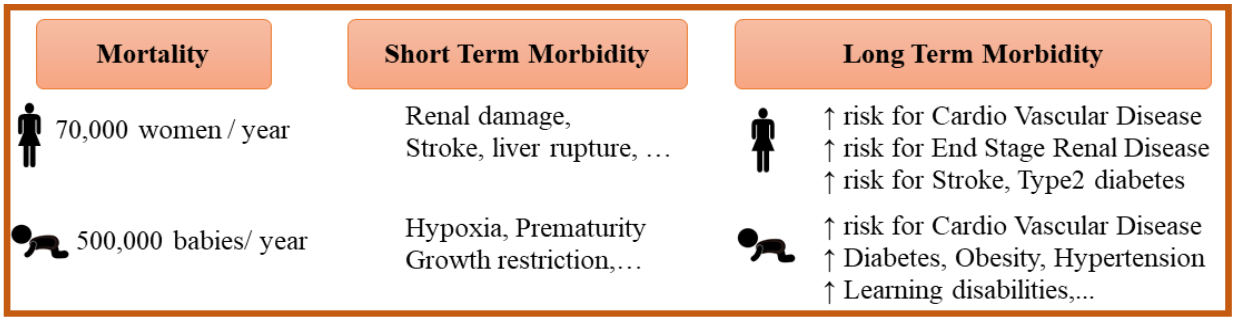
A pregnancy-screening test for preterm pre-eclampsia involving the determination of proteins and metabolites in the same blood specimen, was, and is, acceptable by clinical laboratory operators. With the protein of interest, Placental growth factor (PlGF), widely available in the clinical laboratory market place, using established IVD analyser technology (Roche, PerkinElmer, Siemens, etc), clinical laboratories were and will be supportive to implement Metabolomic Diagnostics 's LC-MS methodology to analyse the metabolites of interest to achieve clinically relevant prediction. In this context, there is, at the end of the IMPROvED Project, no impediment to combine proteins and metabolites in deploying Metabolomic Diagnostics's test PrePsia™.

2.2 Potential Impact

2.2.1 Socio-economic impact

These prototype screening tests assessed and refined in IMPROvED can significantly reduce the burden on individual patients as well as burden to society (healthcare costs) of a major healthcare problem: pre-eclampsia. The screening test, PrePsia™, is now being prepared for clinical validation and industry partner on this study, Metabolomic Diagnostics was awarded €2 million in EU Funding under the latest Horizon 2020 SME Instrument call in March 2018 as part of a plan to introduce PrePsia™ into the European market and beyond.

Pre-eclampsia is the most important cause of maternal death in Europe, accounting for 12-24% of all maternal deaths[17] and globally, causes 70,000-80,000 maternal and over 500,000 infant deaths annually, and 20% of neonatal intensive care unit (NICU) occupancy and costs.



Of the estimated 50 million babies that are born to first time mothers worldwide every year, 2.4 million are in the 27 EU countries.[1] Almost 1 in 20 of these pregnancies are complicated by pre-eclampsia, a disease of late pregnancy characterized by the concomitant occurrence of hypertension and proteinuria.
For the mother it can lead to acute problems in the liver, kidneys, brain and the clotting system, and pre-eclampsia is the most important cause of maternal death in Europe – accounting for 17-24% of all maternal deaths.[17] Additionally, epidemiological studies have demonstrated that pre-eclampsia is associated with an increased risk of cardiovascular and metabolic diseases later in the mother's life.[18] [19] A history of pre-eclampsia increases cardiovascular risk by two to four times, which is comparable to the risk of cardiovascular disease caused by smoking.[20]

A quarter of the babies born to mothers with pre-eclampsia are growth restricted and a third are premature; pre-eclampsia accounts for occupancy of approximately 20% of neonatal intensive care unit costs. The child may have problems with neurocognitive development that can result in mild learning difficulties through to severe disabilities. Being born growth restricted also predisposes the child to cardiovascular disease (high blood pressure, heart attacks and diabetes) as an adult.

Through the established mechanism of the developmental origins of adult disease,[21] the consequences of major pregnancy complications such as Pre-eclampsia are not limited to early life. Offspring who survive an episode of pre-eclampsia are at much greater risk of cerebral palsy and neurodevelopmental delay, and have an increased risk of non-communicable diseases (NCDs) such as obesity, cardiovascular disease, hypertension, diabetes and schizophrenia in adulthood.

Indeed, up to 50% of the risk of NCDs has been attributed to early life events. Global rates of these conditions have increased rapidly and impose massive burdens on public health systems, the economy and society. For example, obesity in Europe is increasing at an alarming rate. Over the last decade the proportion of the population that is overweight has increased considerably in most member states, resulting in more than half the EU population being overweight or obese (Eurostat). There are 60 million people with diabetes in Europe, and each year cardiovascular disease causes over 1.9 million deaths in the EU (WHO, European Heart Network). In fact, epidemiological studies

have demonstrated that pre-eclampsia is associated with an increased risk of cardiovascular and metabolic diseases later in the mother's life.[3] Prevention of these health problems is of paramount importance to future mothers, fathers and children. This clinical use of this test early in pregnancy has the potential to impact positively on the 2-3 million cases annually of first time pregnancies complicated by pre-eclampsia.

2.2.2 Health Economic Impacts

Compelling health economic arguments: Every year, an estimated €31 billion is spent in the developed world on direct healthcare costs to provide antenatal care for nulliparous women and treatment for pre-eclampsia; of this, an estimated €9 billion is spent in Europe.[22]

The average cost of antenatal care (UK model - 13 visits) is €3,800;[22] an effective screening test would facilitate stratification and targeting of limited resources. The healthcare costs of one case of pre-eclampsia are estimated to exceed €15,000 (all maternal and neonatal hospital costs – but not accounting for long term implications for the baby).

Therefore, preliminary probabilistic sensitivity analyses suggests that even on assumptions that an effective test only halves antenatal visits, and the administration of therapies such as aspirin reduce incidence of disease by only 20-25%, potential savings would approximate to €3-4 billion of the estimated annual €9 billion spent in Europe to provide antenatal care for nulliparous women and treatment for pre-eclampsia. Preliminary probabilistic sensitivity analyses suggest significant economic benefit even if the unit cost of a screening test was €400- €700.

Stakeholder Impact Summary

Stakeholder	Impact	Measurable Outcomes
Participant SME's	Delivery of a technically and commercially viable pre-eclampsia risk screening test to the market.	Company Revenue Growth in Employee numbers
Mother & Baby	Mothers who are identified as high risk are more attentive to clinical symptoms. This will help women in the early recognition of potential symptoms, and hence empower pregnant women to get more timely access to the appropriate care. Mothers at high risk are monitored more closely and have personalised care designed to reduce bad outcomes.	Reduction in mortality and morbidity rates Healthier Baby. Healthier Mother. Reduced length of hospital stays.
Clinicians	Knowing the risk profile of their patients, clinicians can initiate personalised preventative treatment such as Aspirin to the high-risk group. Clinician's time is more targeted to those at high risk while maintaining a level of care to those at low risk.	Reductions in - Bad outcomes - Emergency care - Caesarean sections - Intensive care
Healthcare Payers	Identification of pregnancies at high risk of pre-eclampsia early in pregnancy enables appropriate pregnancy management which will lower the number of high cost adverse events, hence reducing cost of care overall.	Optimised prenatal care programmes. Reduced cost of care for pre-eclampsia and its consequences.
Pharma Companies	**PrePsia™** will support new intervention therapeutics through identification of at risk pregnancies for clinical trials and future clinical intervention.	Growing support for the development of novel therapeutics for pre-eclampsia

Impact in the relevant industry (in particular for SMEs)

The project is a key enabler for the SME Metabolomic Diagnostics to deliver a commercially viable pre-eclampsia risk stratification product, PrePsia™, to the market. PrePsia™ will identify those women who are at risk of developing preterm pre-eclampsia later in their pregnancy. Metabolomic Diagnostics market research has identified the 10 target markets which represent the most appropriate and receptive markets to initially launch PrePsia™ in. These markets represent 8.5million pregnancies. Adoption of the test in these 10 initial markets will save many lives. Identifying the women at risk of developing pre-eclampsia supports intervention by clinicians which can improve pregnancy outcomes for those women and their babies. Success in these initial markets for Metabolomic Diagnostics will create jobs within the SME as well as creating a solid market foundation for the development of future products. Within 4 years Metabolomic Diagnostic's will employ at a minimum 50 people. The pre-eclampsia test market is a global opportunity, a clearly defined unmet clinical need, success in this field will ensure Metabolomic Diagnostics is a leading light in pregnancy diagnostics using innovative biomarker techniques. World attention will be focused on the first truly significant translation of a research project in the area of metabolomics/proteomics, and successful application of the discovery-validation pipelines in other disease areas would be highly likely. This presents Europe with the opportunity to establish its credentials as a centre of excellence in developing the next generation of diagnostics, thus attracting foreign direct investment from other companies active in this space.

Metabolomic Diagnostics has already closed 4 successful VC investment rounds. The first two in 2013, 2014 raised a combined 1.5m. In 2016, the company closed an investment round of €1.65M and in early 2018 an additional €1,0m, for a total of €4.15m.

Impact for the scientific community via Training, Dissemination and Education

It is vitally important that the progress and results of the IMPROvED study are effectively disseminated to the wider scientific community. Raising awareness of the project and its aims will pave the way for a positive introduction of the preclampsia tests when they come to market. In addition, building a profile for the IMPROvED biobank and making the scientific community aware of the potential to access IMPROvED samples and data will foster collaborations and encourage researchers to obtain funding and ethical approval for evidence based studies they would not normally be in a position to perform because of lack of access to large patient cohorts. To ensure the effective dissemination of the work of the consortium a publication committee has been formed and a publication policy put in place.

A dedicated project website was set up and launched in time for the kick-off meeting in November 2013 at the following URL: http://fp7-improved.eu/. The publicly accessible pages were designed to inform the general public as well as the scientific and political communities about the IMPROvED project, pre-eclampsia, risk factors, treatment and the value of rapid diagnosis. The website was a key dissemination channel, serving as a comprehensive source of information for external parties with an interest in the work done within the IMPROvED consortium. The main objectives were to disseminate information about the project's activities and results as widely as possible and to make it easy to access by the various target groups such as decision makers, pregnant women and healthcare stakeholders having interest in the project's scientific and technical results. As such, http://www.fp7-improved.eu/ aimed to go beyond a purely descriptive scientific project website and build a virtual hub for the pre-eclampsia community. Accelopment AG was in charge of the creation, updating and maintenance of the project website throughout the entire course of the study.

The website included an overall description of the project (objectives, structure, innovations, consortium, expected impact), as well as listings of planned events and activities carried out by the partners of the project. In accordance with the objective to recruit 5000 first time mothers, over 2 years, to academic medical centers across Europe, there was a designated section for expecting mothers, which provides information on pregnancy problems, invitation to join the study and lists the recruitment centres. The website features a news plugin to organize and display news articles in

selected pages / frames and a links to the FaceBook page and Twitter account that were set up for the Project.

Visitor traffic has been monitored with Google Analytics. Throughout the project a total of 10,086 users visited the IMPROvED website. Those users had a total of 32,283 sessions with a total of 52,627 page views. About 26% of users are from Ireland, followed by visitors from the United Stades (12%), the United Kingdom (10%) and the Netherlands (6.5%). The most popular pages on the website, apart from the homepage, were as already in previous periods the information page on the recruitment centres, the projects objectives and in third place the 'Your participation' page. Given that the public's participation is vital for this project, it is a pleasing development that these pages had the most visitors. 44% of all sessions started through a search on a search-engine. 34% of all sessions were accessed directly (by entering the URL and not going through search), 19% of all session were started by a referral from another website and 3% of the sessions were started from links shared on social media.

Social media presence (Facebook and Twitter), has solidified throughout the project duration. The Facebook Page (www.facebook.com/IMPROvED.FP7) above all has proved instrumental in reaching our target audience, keeping up the public interest in the study and aiding the recruitment process. The Facebook community has currently 1,162 members (status: 16 April 2018), with a typical organic post reach (the number of people a post was served to) between 400 to 1,200 and approximately 40 weekly page views. On average, the page posts at least once every week, on issues related to recruitment to the study, on the disorder of preeclampsia, on pregnancy in general and news related to our project partners and IMPROvED. The Twitter account is being maintained by IMPROvED study midwives and the Facebook page jointly by staff at UCC (research) and accelopment (communication).

To ensure consistency across documents produced by various partners and to ensure uniform recognisable messaging occurred, Accelopment AG provided the Consortium with template documents such as the Corporate Design Manual, PowerPoint presentation template, Time sheets template (Excel), Progress report template (Word), Minutes template (Word), Meeting agenda template (Word), Deliverable template (Word) to provide a recognisable IMPROvED Corporate Identity. This Corporate Identity was also used to assist in the recruitment process with template recruitment posters produced that were translated in recruitment site-specific languages. These products were widely used by all recruitment centres.

2.2.2.1 Patient Advisory Board

IMPROvED's dissemination strategy aimed to raise awareness of the IMPROvED project through interaction with patient networks, clinicians, funding agencies and the scientific community worldwide. A central part of this strategy was communication with patient groups particularly active engagement with our Patient Advisory Board (PAB). The PAB plays a key role in ensuring that the IMPROvED consortium interacts optimally with the wider community. PAB members are acutely aware of the needs of the patient and this input is invaluable in ensuring that the IMPROvED programme is relevant to pregnant women across the different European jurisdictions.

2.2.2.2 Publications and Publicity

The consortium is conscious that many of the publications associated with IMPROvED will only be possible towards the latter stages of the project when results are available. With the extension of the recruitment phase, this delivery period is also being further delayed. The Publication Committee and representatives of several IMPROvED consortium partners held a teleconference to discuss plans for publishing, with the following topics identified:

- Clinical risk prediction: 3 papers.
- SMEs will have PET assay publications.

- METABOLOMIC DIAGNOSTICS and RUG suggested to write a joint paper on modelling.
- NICE (National Institute for Health and Care Excellence, UK) compliance paper.

Peer-reviewed publications

A multi-centre phase IIa clinical study of predictive testing for pre-eclampsia: improved pregnancy outcomes via early detection (IMPROvED). Navaratnam K, Alfirevic Z, Baker PN, Gluud C, Grüttner B, Kublickiene K, Zeeman G, Kenny LC. BMC Pregnancy Childbirth. 2013 Dec 7;13:226. doi: 10.1186/1471-2393-13-226

Pre-eclampsia Diagnosis and Treatment Options: A Review of Published Economic Assessments. Zakiyah, Neily et al., on behalf of the IMPROvED Consortium. PharmacoEconomics 2015:291. Published online: 06 Jun 2015; doi: 10.1007/s40273-015-0291-x

Early Pregnancy Biomarkers in Pre-Eclampsia: A Systematic Review and Meta-Analysis. Wu, Pensée et al. Int. J. Mol. Sci. 2015, 16(9), 23035-23056. DOI: 10.3390/ijms160923035

Published Abstracts

A multi-centre phase IIa clinical study of predictive testing for pre-eclampsia. IMproved PRegnancy Outcomes Via Early Detection (IMPROVED). Kenny L; IMPROVED Consortium. Pregnancy Hypertens. 2013 Apr;3(2):60. doi: 10.1016/j.preghy.2013.04.011

Pre-eclampsia risk stratification early in pregnancy: Levering a promising metabolomics discovery in a LC–MS based clinical assay. Bond, Liz et al. In: Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health, Volume 5, Issue 1, 82. January 2015; doi: 10.1016/j.preghy.2014.10.164.

Oral Presentations

European Congress of the ISSHP (International Society for the Study of Hypertension in Pregnancy), Tromso (Norway), 12 June 2013

> Title: The IMPROVED consortium and biomarkers for the prediction of pre-eclampsia. A multi-centre phase IIa clinical study of predictive testing for pre-eclampsia. IMproved Pregnancy Outcomes Via Early Detection (IMPROvED).
>
> Presenter: Prof Louise Kenny (UCC), on behalf of the IMPROvED Consortium (Appendix I)

Regional Consortium Obstetrics & Gynaecology Rotterdam (The Netherlands), 15 April 2014

> Title: the IMPROvED-study
>
> Presenter: Drs. Caroline van den Berg (EMC)

Action on Pre-eclampsia Expert Meeting 2013 London (United Kingdom), 6 November 2013

> Title: IMPROvED- Personalised medicine for pregnant women, metabolomic and proteomic biomarker research
>
> Presenter: Prof Phil Baker

7th Congress of the International Society of Gender Medicine and to the International Congress of Gender Medicine of GIM (Institute of Gender in Medicine) at Charite that are held in close cooperation, from September, 20-21 and 22-23, 2015, in Berlin, Germany. EU-project IMPROvED: (IMproved PRegnancy Outcomes by Early Detection, presented by K. Kublickiene

Poster Presentations

ESBB (European, Middle Eastern and African Society for Biopreservation and Biobanking) Verona (Italy), October 2013

Title: Ensuring Quality within the EU-FP7 IMPROvED Biobank.

Presenter: Emma Snapes, UCC

AUDGPI (Association of University Departments of General Practice in Ireland) Annual Scientific Meeting, Cork (Ireland), March 2014.

Title: IMPROvED Study

Presenter: Nicolai Murphy, UCC

NFOG congress (Nordic Federation of Societies of Obstetrics & Gynaecology) Stockholm (Sweden), 10-12 June 2014

Title: A multi-centre phase IIa clinical study to develop a predictive blood test for pre-eclampsia – IMproved PRegnancy Outcomes via Early Detection (the IMPROvED-study).

Presenter: Boel Niklasson, Karolinska Institutet

Society Maternal Fetal Medicine Global Annual Meeting, Meeting of the Pre-eclampsia Biomarkers Consortium, February 2016, Atlanta, Georgia/USA.

MEDTEC Ireland October 2016, Galway, Ireland

Title: Pre-eclampsia Case Study Presentation

Presenter: Diarmuid Cahalane

ISSHP 2016, 24/10/2016. Sao Paulo, Brazil.

Title: Oral Presentation on IMPROvED

Presenter: Louise Kenny

“Pre-eclampsia and other major pregnancy complications” at the Gates Foundation Grand Challenges Annual Meeting, London, UK.

National and International press:

Public awareness is critical to encourage recruitment and to raise the profile of IMPROvED, ProTest and MetTest. At each recruitment centre press releases were issued at the start of recruitment and the first recruits were featured on the IMPROvED website as well as on the study’s Facebook page. Interviews with local radio and TV stations were also conducted.

UCC organised a media launch to coincide with enrolment which featured photos of the first recruit and her husband with IMPROvED research midwives. The story was covered in national and local newspapers.

28/4/2014 – Irish Times – Science Foundation Ireland: Search is on to find screening test for pre-eclampsia

3/7/2014 – Medical Independent - Pre-eclampsia Awareness Initiative Launched

31/8/2014 – Irish Examiner - Pre-eclampsia awareness is important for pregnant women

Since the launch of IMPROvED the study has been reported in a number of patient and professional publications such as the Spring edition of Oh Baby! Magazine and the Irish Medical Times

1/3/2015 – Mums & Tots Magazine – All About Pre-eclampsia

1/3/2015 – Mums & Tots Magazine – Early Prediction of Pre-eclampsia

27/5/2015 – Irish Examiner – Potential Game-Changer for Expectant Mums

30/9/2015 – Irish Examiner – UCC Project to Help Expectant Mums

8/10/2015 – Medical Independent – The Mother of Invention – Making Maternal Healthcare Matter

Irish Times: 19/01/2016, Tissue storage could help to identify risks of pre-eclampsia http://www.irishtimes.com/life-and-style/health-family/tissue-storage-could-help-to-identify risks-of-pre-eclampsia-1.2495432

Irish Examiner: 19/08/2016, UCC biobank to help improve health of mothers and babies http://www.irishexaminer.com/ireland/ucc-biobank-to-help-improve-health-of-mothers-and-babies-416633.html

In August 2016, the Irish Examiner published an article on the new UCC/INFANT biobank, where among others, the IMPROvED samples are being stored and which serves as a basis for the screening test towards which the research is aimed. IMPROvED Coordinator Prof Kenny and Quality Committee member Emma Snapes (in their capacities as director respectively quality and regulatory manager of INFANT) were both interviewed for this article.

18/11/2016 – INFANT Helping to make pregnancy, birth safer. National Newspaper.

The IMPROvED study was published in two Dutch newspapers (“Algemeen Dagblad and “de Havendloods”, EMC also organised a media launch with a photo session with the first local recruit and reported in VOG magazine, April 2014. There was a radio interview on the local radio station (Rotterdam Rijnmond) at the 1st of April 2014.

Karolinska Institute published an IMPROvED advertisement in six local newspapers (www.mitti.se) and in the Karolinska Institutet newspaper (“KI Bladet”) which included a photo session with the first local recruit. IMPROvED was presented at a Women’s Health Day, a public event in Stockholm in September 2013 and also the Nobel Forum: Sex, Drugs and Medical Devices III, Karolinska Institutet (Solna, Sweden) in October 2013.

22/5/2017 - HORIZON The EU Research & Innovation Magazine - Humble aspirin helping solve one-in-20 pregnancy threat (Interview with Nicolai Murphy).

22/05/2017 – Pre-eclampsia: The condition that kills 100,000 pregnant women. Irish Examiner.

25/09/2017 - Award-winning Irish researcher investigating treatment for common pregnancy complications. Breakingnews.ie

Local Newspapers

4/07/2014 – The Cork News – Drive for Pre-eclampsia Awareness

27/10/2014 – Evening Echo – INFANT aims to help overcome pregnancy difficulties

29/01/2015 – Evening Echo – UCC Study Set to Books Pregnancy Outcomes

07/09/2015 – Evening Echo - Pregnancy Research in Cork wins International Award (this article featured in the Kildare Nationalist, Laois Nationalist, Rosscommon Herald, the Nationalist, Waterford News & Star, and the Wexford Echo).

08/09/2015 – Evening Echo – Pregnancy Research in Cork Wins International Award (also featured in the East Cork Journal and Cork Independent)

23/09/2015 – Evening Echo - Cork is Leading the Way in Pregnancy Research Efforts

29/09/2015 – Evening Echo – Cork Parents are So Amazing, Without Them We Couldn't Do This Research

30/09/2015 – Evening Echo – Project to Make Pregnancy Safer

4/11/2015 - Evening Echo – Top Award for UCC Researchers Working to prevent Diseases in Pregnancy

11/02/2016 – Cork Independent – Mum's the World – We've Hit 1000! http://www.corkindependent.com/news/topics/articles/2016/02/11/4114237-mums-the-word--weve-hit-1000/

25/05/2017 – Evening Echo – Raising Awareness About Pre-eclampsia

15/11/2017 – Cork Independent – Spotlight on Pre-eclampsia

Television

In October 2016, for a TV programme called 'Big Data', an IMPROvED visit and PI discussion was filmed, to air during Science Week in November 2016 on RTE, the Irish national television station.

17/11/2016 - Cloud Control: Who Owns Your Data (RTE1)

18/11/2016 – Cloud Control: Who Owns Your Data (Cork Film Festival)

RADIO

The Global Clinical Coordinator, (Nicolai Murphy) was interviewed on an Irish national radio station (RTE Radio 1) during the prime time morning slot on the 29th November 2013 and on the national TV station (RTE 1) Morning Edition show.

08/09/2015 – Newstalk FM Pat Kenny Show – Interview with Louise Kenny

09/09/2015 – Shannonside Radio – Joe Finnegan Show – Interview with Nicolai Murphy

09/09/2015 – CRY Radio - Interview with Nicolai Murphy

10/09/2015 – Kildare FM - Interview with Nicolai Murphy

In September 2016, Prof Kenny was interviewed on the "Opinion Line" talkshow of Cork's 96fm radio station about pre-eclampsia and other pregnancy complications, the importance of screening tests, the IMPROvED study and how to get involved in it. The audio recording (podcast) is available on soundcloud.com.

Internet Based News

02/12/2013 – Chemical & Engineering News: Early Detection Of Pre-eclampsia https://cen.acs.org/articles/91/i48/Early-Detection-Pre-eclampsia.html

03/12/2013 - New Project Offers Clear Hope for the Early Detection of Pre-eclampsia https://www.ucc.ie/en/med-health/news/new-project-offers-clear-hope-for-the-early-detection-of-pre-eclampsia-.html

03/07/2014 – Pre-eclampsia Foundation - Ireland Declares First National Pre-eclampsia Awareness Day: https://www.pre-eclampsia.org/es/representacion-del-paciente/archivo/377-ireland-declares-first-national-pre-eclampsia-awareness-day

03/07/2014 - The Journal - Pre-eclampsia kills 76,000 pregnant women each year but warning signs can go unnoticed

27/03/2015 – Silicon Republic - Cork-based professor looks to innovation to make pregnancy safer

01/08/2015 – Oh Baby Magazine - First Pregnancy? Improved wants you!

03/09/2015 – HerFamily – First Pregnancy? Join This Study

07/09/2015 – Silicon Republic - Prof Louise Kenny wins international award for INFANT work

07/07/2015 – Mummy Pages - International honour awarded to UCC and Cork University Maternity Hospital

07/07/2015 – Media HQ - Pregnancy research in Cork wins international award

09/12/2015 – Silicon Republic - Science Squad and Prof Louise Kenny recognised at European science media festival

http://www.metabolomicdiagnostics.com/mother-of-invention-making-maternal-care-matter/

EPE Events

22/05/2017 – World Pre-eclampsia Day Events @Cork University Maternity Hospital

22/05/2017 – Facebook Video on IMPROvED social media on the IMPROvED Study

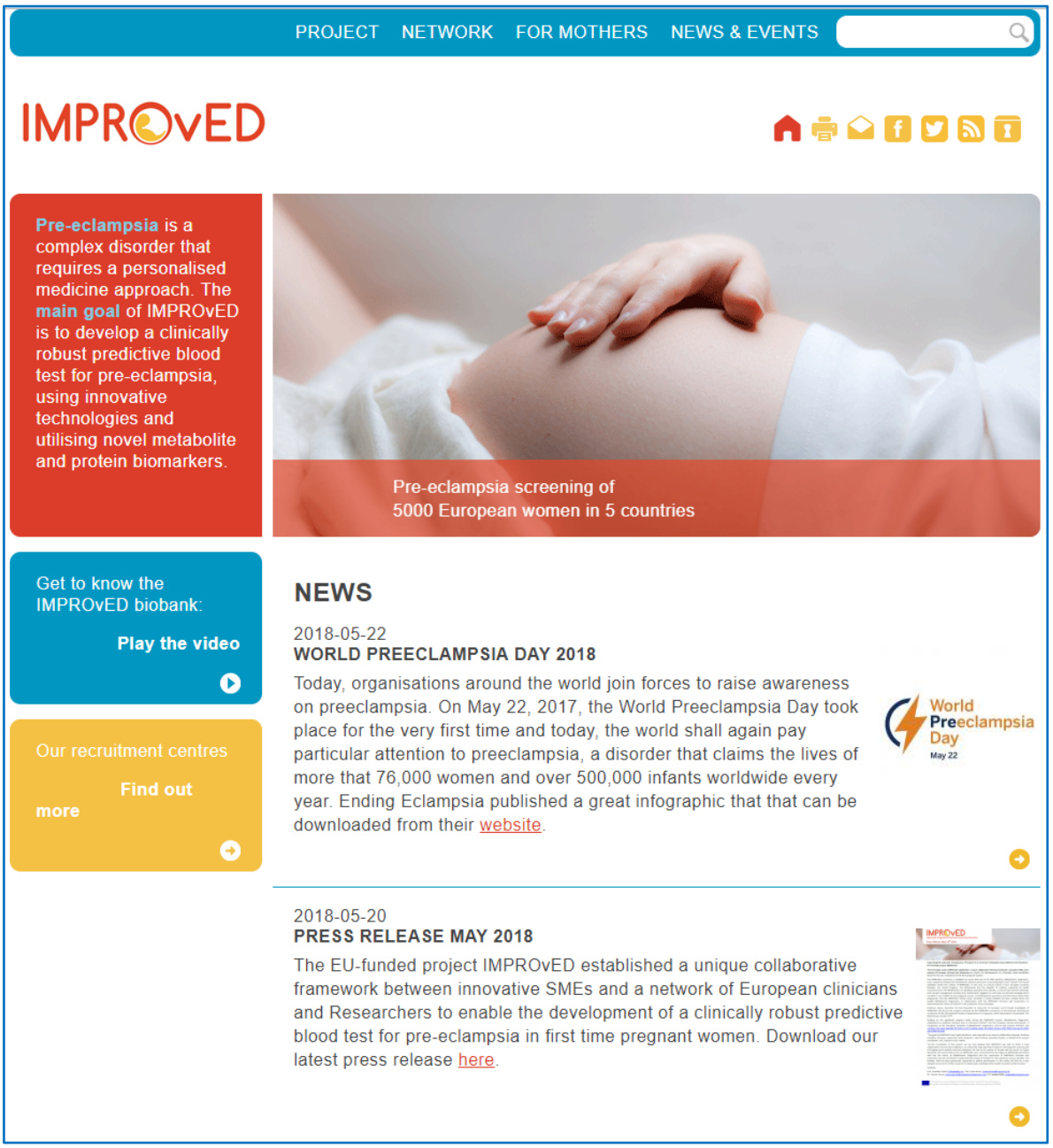
01/09/2015 - Development of a primary school STEM workshop based on the IMPROvED study, which, over 3 years has been delivered to over 2000 primary school children.

A list of publications and conference presentations, complete with download links where available, is available on the project website at www.fp7-improved.eu/project/publications.

All dissemination activities lead to greater awareness of the risks of pre-eclampsia and foster a culture where open discussion is encouraged. Members of the Patient Advisory Board have reported that in certain instances maternity care givers do not discuss pre-eclampsia with pregnant women as they feel it will frighten/stress the women. However, other members have reported the effectiveness of open discussion and in the Netherlands all pregnant women are supplied with a leaflet from the Dutch patient group containing pre-eclampsia information. In addition, the availability of a reliable predictive screening test early in pregnancy should encourage maternity care givers to speak more freely about pre-eclampsia.

In order to get a good indication of what constitutes care as usual for pregnant women in the different participating countries, a web-based survey was developed for health-care workers. It contains questions on the management of healthy pregnancies, pregnancies considered at high-risk for developing pre-eclampsia, and pre-eclampsia pregnancies. In December 2015, the web-based survey was disseminated amongst the IMPROvED partners for further diffusion within their networks of healthcare professionals. Obstetricians and midwives from 5 countries (Sweden, the Netherlands, the UK, Germany, and Ireland), filled out the survey, providing a wealth of background information on clinical practice around pregnancy in general and pre-eclampsia in particular.

2.3 Public Website and Contact Details.



http://www.fp7-improved.eu/

https://www.facebook.com/IMPROvED.FP7

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