IMI1 Final Project Report Public Summary

Project Acronym: SUMMIT

Project Title: SUrrogate markers for vascular Micro- and Macrovascular hard endpoints for Innovative diabetes Tools

Grant Agreement: 115006
Project Duration: 01/11/2009 - 31/10/2015

Executive summary

1.1. Project rationale and overall objectives of the project

Diabetes mellitus is a lifelong, incapacitating disease affecting multiple organs, which at present cannot be cured but only symptomatically treated or, at best, partially prevented in the case of type 2 diabetes (T2D). The disease is associated with devastating chronic complications including coronary heart disease, stroke and peripheral vascular disease (macrovascular disease) as well as micro-vascular disorders leading to damage of kidneys (diabetic kidney disease or DKD), eyes (retinopathy or DR) and feet (lower extremity arterial disease or LEAD). These complications impose an immense burden on the quality of life of the patients and account for more than 10% of health care costs in Europe. Novel means to prevent and/or treat these devastating diabetic complications are urgently needed. SUMMIT aimed to accelerate the development of such therapies.

SUMMIT ***… AIMING FOR ACCELERATED PROGRESS***

There is an expanding therapeutic need for better diagnostics and new treatments of DIABETIC COMPLICATIONS beyond existing – mostly glucose lowering - therapies.

Clinical trials to show benefit of such therapies are large, long-lasting and costly.

The key objective of SUMMIT was the DEVELOPMENT OF KNOWLEDGE, PROCEDURES, TECHNOLOGIES AND TOOLS to make clinical trials testing of novel medications in diabetic complications shorter and more focused.

Key INDICATIONS: Diabetic Kidney Disease (DKD), Diabetic Retinopathy (DR) in T1D and T2D and Cardiovascular Disease (CVD) in T2D patients.

1.2. Overall deliverables of the project

SUMMIT developed innovative approaches to make clinical trial testing of novel medications in diabetic vascular complications faster and more efficient. To do so SUMMIT aimed to develop novel **genetic markers** and **soluble biomarkers**, which can be used i) to identify patients at high risk (antecedent biomarkers), ii) differentiate between fast-progressors and slow-progressors and iii) monitor progression, reduction or prevention of diabetic vascular complications. In addition, SUMMIT aspired to develop novel and improve existing **imaging techniques** for monitoring progression in atherosclerosis and retinopathy, novel **animal models** for micro- and macrovascular complications to better replicate disease as seen in man and novel ***in silico* methods** for modelling and predicting diabetic complications. The ultimate goal still is to make these markers and tools accepted by regulatory authorities and to disseminate the findings not only to the scientific community but also to lay people and patient organizations.

SUMMIT ... *DELIVERABLES WITH IMPACT*

Susceptibility markers and tools predicting diabetic complications:

- ***Novel GENETIC MARKERS / BIOMARKERS / IMAGING MARKERS that can be used to***
  - ***identify patients at high risk of complications***
  - ***differentiate between fast-progressors and slow-progressors***
  - ***monitor progression, reduction or prevention of complications***
  - ***serve as useful surrogate endpoints in clinical trials***
- ***Better ANIMAL MODELS / DATA MINING AND IN SILICO MODELLING TOOLS / IMAGING TECHNIQUES***

1.3. Summary of progress versus plan since last period

The main focus during the final year of SUMMIT has been on completing the DKD and CVD work, where SUMMIT has its exceptional strength. Yet also considerable effort has been put into finding new surrogate markers for DR and LEAD.

The **follow-up genotyping and the validation studies for the genetic biomarkers** have been completed, and the data analyses have identified some promising **genome-wide hits for DKD** and several **hits for interactions relating CAD or peripheral vascular disease** with diabetes status. Work will continue after SUMMIT to gain more statistical strength especially for genetic markers for DR and LEAD.

The **SUMMIT-JDRF collaboration**, integrating SUMMIT's DKD genetics efforts with those supported by the JDRF, has progressed well, phenotypes are aligned for the genome-wide association study (GWAS) and whole exome sequencing (WES) analyses and analysis plans are prepared. Some delays in the availability of the JDRF-DNCRI data will extend the completion of these collaborative analyses past the final date of SUMMIT, as agreed with the JDRF.

The remaining **non-genetic biomarker validation studies for CVD and DKD in T2D individuals** have been completed. This required measurement of biomarkers, identified during the discovery phase, in new sets of samples supplied for validation. A SUMMIT-wide collaboration explored the analysis of the **genetics of the biomarkers** for DKD and CVD.

The **patent application** for the panel predicting rapid decline in renal function in diabetes, submitted in March 2014, was updated to incorporate the results of the latest analyses. The patent is at present under review.

The **3-year follow up investigations** of the SUMMIT macro-vascular imaging and the retinopathy OCT studies have both been completed according to plan. Several papers on the baseline investigations of the studies have already been published and additional publications are in preparation. A paper based on the description and validation of the newly developed UPSA technology imaging technology for non-invasive assessment of atherosclerotic plaque structure is in revision for *Atherosclerosis*.

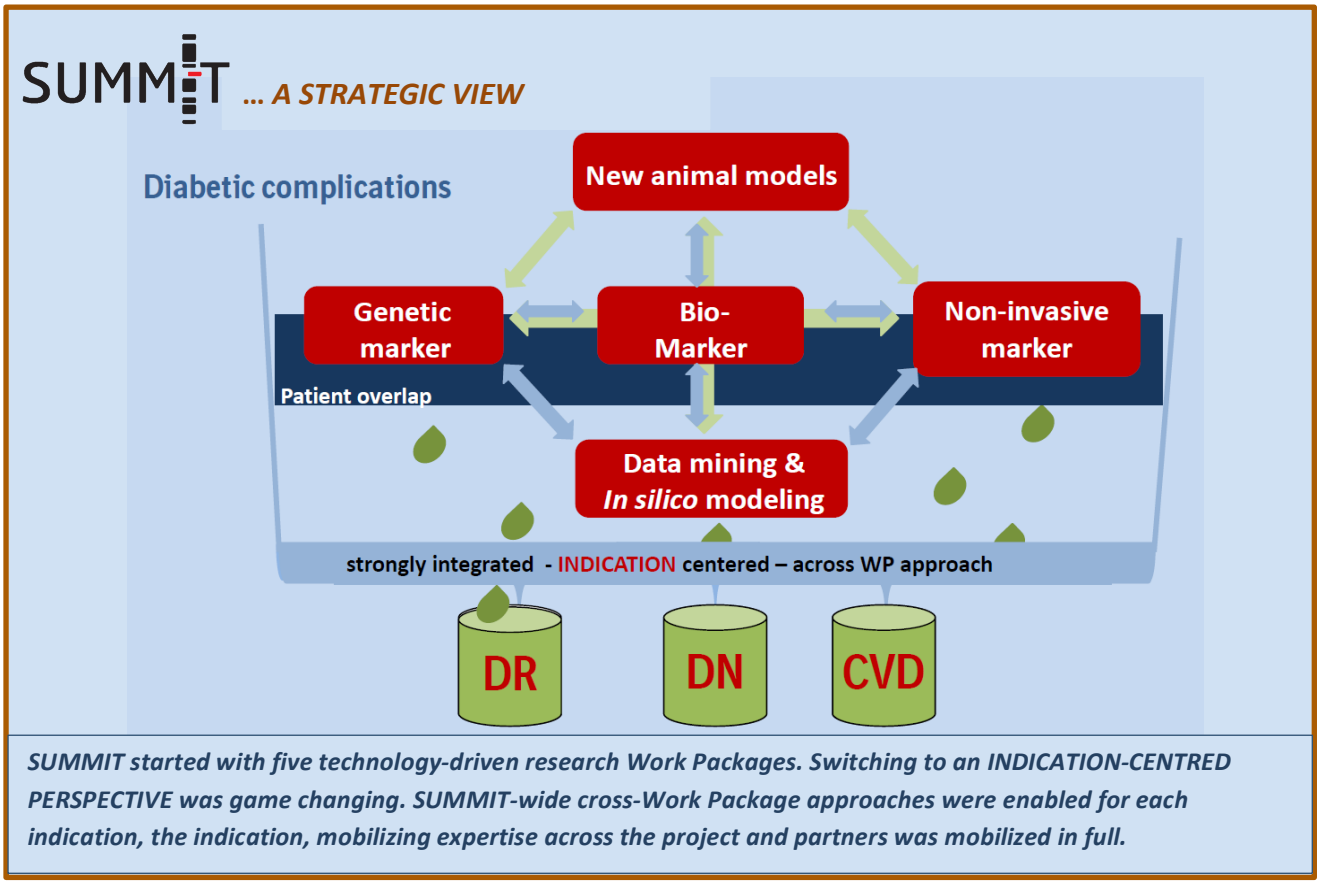
The **ENSO drug intervention studies** in selected SUMMIT animal models were successfully conducted and reports completed for all 4 models. Biomarkers were measured in our ENSO rat models and stored samples open up for future analyses. Goals for the characterization of the for **SUMMIT generated animal models**, among which the KO mice of candidate genes for DKD crossed with the diabetic Akita mouse model and the SUR1xIGFII and SUR1xLDLR KO mice, were reached. Previous reports on **functional stiffness parameter analysis** in animal models were complemented with new data.

The *in silico* model on the **long-term simulation of clinical complications** in type 1 diabetes (T1D) was published.

SUMMIT organized a highly appreciated **open SUMMIT symposium**, in connection to its final Plenary meeting, to celebrate the collaboration and its achievements.

1.4. Significant achievements since last report

The research focus was set again on the different diabetes complications, instead of staying with the more technology-driven division of the WPs and as implemented starting halfway through the SUMMIT project, continued to be a fruitful and inspiring approach.



SUMMIT started with five technology-driven research Work Packages. Switching to an INDICATION-CENTRED PERSPECTIVE was game changing. SUMMIT-wide cross-Work Package approaches were enabled for each indication, the indication, mobilizing expertise across the project and partners was mobilized in full.

SUMMIT completed all its IMI-supported tasks, including the ENSO drug intervention project, which started in the second half of period 5, and we progressed well in the SUMMIT-JDRF collaboration.

Completion of the extensive and logistically demanding ENSO studies within the limited allocated time has been the major achievement since the last report, providing evidence of a well-functioning consortium

Diabetic Kidney Disease (DKD)

We identified a promising locus from the **micro-albuminuria phenotype** in subjects with T2D that may also represent an eQTL for Gamma-aminobutyric acid (GABA) B receptor 1 (GABR1). We have also demonstrated that the uromodulin (UMOD) variant association with DKD extends to diabetic kidney disease, in subjects with either T1D or T2D. A manuscript describing the T1D findings is well advanced, and a second describing the T2D results will follow further attempts at replication.

Genetic and epigenetic factors influencing the diabetic kidney were identified: effects on osteopontin (OPN) expression (Cai et al 2015), on the Thioredoxin-interacting protein (TXNIP) gene (de Marinis et al, in press) in T2D patients, as well as Kidney injury molecule-1 (Panduru et al 2015) and factors affecting mitochondrial function (Swan et al 2015).

As part of the **SUMMIT-JDRF collaboration**, we have harmonized the phenotypes for the GWAS and WES analyses and have prepared analysis plans for both aspects of the genetic data so that we may proceed with analyses once data are available from the DNCRI. We have applied for and have been awarded a no cost extension for the JDRF grant in order to achieve the aims of the project.

The paper by Looker et al (*Kidney International* 2015) from a SUMMIT-broad collaborative effort describes the discovery phase results for predicting **biomarkers for rapid chronic kidney disease progression in T2D** patients. These data were presented at the European Diabetes Nephropathy Study Group meeting in May 2015 in Copenhagen and at the IMI session at the European Association for the Study of Diabetes (EASD) meeting in Stockholm, September 2015.

This year the **validation studies for the DKD non-genetic biomarker** studies were completed. WP2 and WP5 undertook analyses of the final validation data in close collaboration; manuscripts based on this work are in preparation. We also worked closely with WP1 and WP5 to examine the utility of combining genetic and biomarker data. The integration of genetics with biomarker data did not yet lead to identification of novel causal biomarkers but there are interesting indications of novel genetic hits for metabolites and these studies will be continued.

We successfully developed and characterized two **new diabetic mouse strains** (SUR1xIGFII and SUR1xLDLR KO) with altered insulin metabolism. The Sulfonylurea receptor (SUR) 1 model proved useful for studies on epigenetic modifications in the kidney, summarized in a paper currently in press in Kidney International (De Marinis et al, in press). The intervention studies with standard of care medication in our DKD relevant animal models, the ZSF and the BBDR.cg-lepr.cp rat, were completed and publications are in planning.

The ***in silico* modelling** team developed a Dynamic Bayesian Network model for long-term simulation of clinical complications, both DKD and CVD, in T1D (Marini et al 2015).

Cardiovascular disease (CVD)

SUMMIT's **CVD imaging** study was completed at the centres in Malmö (Lund University), Exeter, Dundee and Pisa and included carotid intima-media thickness (IMT) Pulse Wave Velocity, (PWV), Ankle-Brachial Pressure Index (ABPI), Ultrasound plaque structure and stability assessment (UPSA) and EndoPat measurements. With a 3-year follow-up examination and the samples also analysed for genetic and non-genetic biomarkers, this resulted in an extremely well-characterized clinical cohort for these diabetic complications.

The baseline results from cross-sectional carotid intima-media thickness (IMT) part of the study were recently published, concluding that measures of atherosclerotic burden are associated with clinically manifest cardiovascular disease in T2D (Shore et al 2015). There were only weak associations between change in carotid IMT and development of clinical events during follow up. However, there was a trend towards more events in those with regression, which does not support decreased progression (or regression) of carotid IMT as an optimal surrogate endpoint for reduced risk of cardiovascular events. However, it cannot be excluded that longer follow up periods are required to identify a decrease in cardiovascular events associated with beneficial effects on carotid IMT.

Difficulties in **cardiovascular endothelial function measurements** by means of aligning the ultrasound probe with the artery under an extended period of time were remediated by integrated hardware/software approaches that improved measurement output (Ramalli et al 2015, Ricci et al 2015).

Other imaging studies using mass spectrometry to analyse carbon and nitrogen isotope ratios in human atherosclerotic plaques obtained by carotid endarterectomy from 56 patients showed direct associations between diet and plaque stability (Goncalves et al 2015). Carotid plaques with low elastin content associated with increased risk of ipsilateral stroke (Asciutto et al 2015), whereas carotid plaques with high levels of interleukin correlate with a reduced risk for cardiovascular events (Grönberg et al 2015).

In addition, abnormalities in cutaneous microvascular reperfusion response leading to altered oxygen increase patterns could indicate dysfunctional microvascular autoregulation, which is important to understand target organ damage (Adingupu et al 2015).

The whole body cardiovascular magnetic resonance imaging study in 158 patients by Duce et al (2015) showed differences in correlations between cardiovascular events and global atheroma burden, when comparing diabetics and non-diabetics.

SUMMIT published the results of yet another SUMMIT-wide key effort, describing the **discovery phase results for predicting non-genetic biomarkers** of cardiovascular disease, also this in T2D individuals (Looker et al, *Diabetologia* 2015). The validation studies of these circulating CVD biomarkers were concluded. These principal CVD biomarker efforts based on the analysis of samples from historical cohorts were complemented by measurement of a biomarker panel in the new SUMMIT CVD sample collection obtained in the frame of the above-mentioned macrovascular imaging study. In a recent publication (Goncalves et al 2015) we showed that plasma Matrix metallopeptidases MMP-7 and MMP-12 demonstrated independent association with atherosclerotic burden and prevalence of CVD. We also identified several other markers that predicted incidence of events and most of these were inflammatory markers. In addition, SUMMIT published several papers

on (circulating) biomarkers related to inflammatory processes in atherosclerotic plaques (Edsfeldt et al 2015, Goncalves et al 2015, Silveira et al 2015) and risk prediction of cardiovascular events (Edsfeldt et al 2016, Engelbertsen et al 2015, Rattik et al 2015). Also the work on *in vivo* platelet activation and antiplatelet drug responsiveness continued, resulting in a number of publications (Patrono 2014 & 2015, Zaccardi et al 2015 & 2016). From the SUMMIT genetic biomarker studies of CVD, two loci that interact with diabetes status to modify the risk of CAD and a novel variant for CAD at genome-wide significance were identified. All findings were in individuals with T2D.

The **ENSO animal models** exhibiting cardiovascular phenotypes, the GK.ApoE and the IGF-II$^{-/-}$ ApoB100/100 mouse as well as the BBDR.cg-lepr.cp rat, were further characterized and validated for their use for related indications (i.e. IGFIIxLDLR KO/ApoB100/100 diabetic mice for pathological cardiac hypertrophy; BBDR.cg-lepr.cp rat for studies on intestinal insulin stimulated glucose uptake and microbiota) and intervention studies in these animals are being concluded. A license agreement with Janvier Labs is in preparation for dissemination of the BBDR.cg-lepr.cp rat model.

SUMMIT generated CUG triple repeat RNA binding protein 1 (CUGBP1) transgenic and ANRIL KO **mice** on LDLR KO/ApoB100/100 mice based on SUMMIT GWAS findings. Both were selected based on findings from early GWAS analyses. CUGBP1 may play a role in myotonic dystrophy while SNPs altering ANRIL expression seem to be associated amongst other with coronary artery disease and diabetes.

The SUMMIT review on animal models of diabetic macrovascular complications as key players in the development of new therapeutic approaches was published (Heinonen et al 2015).

Interesting hyperglycemia effects on gene expression in relation to vascular tissues in mice as well as human tissues were described in Daskoulidou et al (2015) and Hien et al (in press).

Diabetic Retinopathy (DR) and Lower extremity arterial disease (LEAD)

We completed the SUMMIT **prospective multi-centre study** to examine whether macular thickness, as assessed by optical coherence tomography (OCT), predicts progression of diabetic retinopathy (DR) in the earliest stages of DR. The study included 1320 individuals with diabetes and either no or mild diabetic retinopathy; clinical follow-up (diabetes retinopathy status) was obtained by hospital records if a participant was unable to return for follow-up assessments. Over the last reporting period data collection and initial data analysis of the primary research question has been completed. GWAS and non-genetic biomarker measurements were performed in all samples from this study.

With respect to peripheral vascular disease we have found a **genomic region** encompassing SNPs showing interaction with diabetes status. Though we did not identify any genome-wide significant genetic hits for LEAD or DR specifically in subjects with either T1D or T2D, we expect to obtain better results from our continued efforts to expand the sample size and increase statistical strength.

Work in the **animal models** demonstrated the role of the Nuclear factor of activated T-cells (NFAT) in the development of diabetic retinal microvascular complications in mice (Zetterqvist et al 2015).

Scientific publications from SUMMIT external collaborations

GWAS data from SUMMIT have been included in additional collaborations, leading to new discoveries in the field of diabetic neuropathic pain (2x Meng et al 2015)

In collaboration with the FP7 ENGAGE project we explored new methods for linking and harmonisation of biomedical and clinical data to enable integrative cross-biobank research (Spjuth et al 2015).

SUMMIT as a whole

SUMMIT completed its designated tasks, which were multiple for this final period, considering the addition of the ENSO and JDRF projects. The latter was granted a non-cost extension. SUMMIT celebrated the consortium, its achievements and collaborative spirit in a highly appreciated open symposium in Malmö, Sweden, at the occasion of the final Plenary meeting.

SUMMIT initiated and organized the IMI Diabetes Platform session at the 2015 EASD in Stockholm, Sweden.

1.5. Scientific and technical results/foregrounds of the project or the SUMMIT lifetime perspective

SUMMIT's genetic and biomarker efforts became only possible through brought access to a number of well-defined **pre-existing cohorts**. In detail, data and samples from the following historical cohorts were used for analyses in the SUMMIT discovery and validation phases (in alphabetical order):

- ASCEND (University of Oxford)
- BENEDICT (Mario Negri)
- CARDS (Pfizer)
- DEMAND (Mario Negri)
- DIREVA (Lund University)
- EUGENE2 (Gothenburg University)
- EuroDiab Family Study (University of Dundee)
- FinnDiane (Folkhälsan)
- FINRISK (THL)
- GoDARTS (University of Dundee)
- IMPROVE (Karolinska Institute)
- MONICA Augsburg (Helmholtz Zentrum Munich)
- KORA (Helmholtz Zentrum Munich)
- National DNA collection (University of Cambridge)
- Nephropathy Family Study (University of Cambridge)
- ORPS (Oxford Regional Prospective Study; University of Cambridge)
- RISC (University of Pisa)
- Scanian Diabetes Registry (Lund University)
- Steno (through Lund University)
- Stockholm 60-yr old (Karolinska Institute)
- VALID (Mario Negri)

In addition, results were replicated in many other historical cohorts available through the wide SUMMIT network of collaborations.

SUMMIT … WHERE WE COME FROM

The first sketch of the SUMMIT project was in July 2008 submitted to the IMI by means of an expression of interest, seven and a half years ago. At the time genetic and non-genetic biomarkers and certain tools were lacking for basically all diabetic complications. Accordingly, SUMMIT proposed to apply respective technologies to specified SUMMIT complications. Later on the view was reversed from a technology to an indication-driven view. A look back at the pre-SUMMIT limitations and opportunities in the fields of vascular diabetic complications:

THE GENETIC COMPONENT: *Despite an overwhelming evidence for a role of genetic factors in influencing individual risk of diabetic complications, success in identifying the variants responsible using linkage- and candidate-gene based approaches had been limited. The first genome-wide association (GWA) analyses came out only in 2007 and 2008 for DKD and CVD and the numbers were rather low. SUMMIT provided a onetime chance to undertake, for the first time in the area of diabetic complications GWA analyses with the scope and power to bring the uncovering of key pathogenetic mechanisms for DKD, CVD, DR and LEAD in reach. Exome sequencing in DKD was not even considered at the start of SUMMIT.*

THE BIOMARKER COMPONENT: *Soluble biomarkers that predict disease and/or response to disease modifying therapy (predictive and surrogate markers) are a key tool for accelerated, cost effective clinical trials but there were only a few accepted surrogate endpoints for trials to prevent diabetes complications (e.g. LDL-cholesterol, and albumin excretion rate being examples) and little agreement on how to best stratify higher risk patients into clinical trials so as to maximize event rates and power and shorten trial length existed. True for all SUMMIT indications, mostly single biomarker candidates were investigated, with validation and comparison of performance against clinical data often not carried out. SUMMIT proposed a much broader effort, unique in size and scope, for the discovery and validation of such soluble biomarkers for DKD, CVD, DR and LEAD.*

THE IMAGING COMPONENT: *Over the years it had become evident that imaging of atherosclerosis must focus on the vascular wall and not the lumen, a concept reinforced by the finding that atherosclerotic plaques giving rise to clinical symptoms have a typical vulnerable structure. Imaging techniques validated for their usefulness as surrogates for cardiovascular endpoints had their limitations when it came to clinical trials, e.g. their invasiveness, the use of ionizing radiation or high costs. Most widely used was the ultrasound analysis of carotid intima media thickness (IMT) and plaques. However, the predictive value was low and long follow-ups were required. The question at hand for SUMMIT was if a more sophisticated analysis of the ultrasound echo by e.g. analysis of the grey-scale median (GSM) could make a difference in CVD clinical trials. Also monitoring the progression of retinal changes in the case of DR was challenging. Typically measured by quantification of vascular morphological changes according to specified diabetic retinopathy scores (DRS), it would be of great interest to find more sensitive ways of measuring retinal changes in diabetes.*

THE MODELS AND MODELLING COMPONENT: *A lack of animal models that can predict the development of diabetic micro- and macrovascular complications was a major issue in the development of novel therapies. It was known that commonly used animal models of diabetes did not reproduce all human vascular complications but a systematic characterization for the presence of diabetic complications in the existing animal models was lacking as were novel, optimized animal models to better reflect the complications as seen in humans. In silico tools optimized to integrate accumulating -omics knowledge into a clinical macro level and to test the effect of novel therapies on diabetic complications in patients were envisioned but not available. Only recently, a large-scale model of glucose metabolism had been accepted by the FDA as a substitute for preclinical studies. SUMMIT was equipped with the expertise to develop such models.*

SUMMIT was expanded from the initially 5- to a 6-year project, mostly to accommodate new research efforts arising from previous SUMMIT findings that won support by the IMI. However, the additional year also helped to better conclude parts of the project's initial activities, raising their quality and impact. SUMMIT has used its time and delivered to the maximum, the best of the diabetes and diabetes complications private and public research communities, the clinicians and the patients. Important achievements were made by advancing our knowledge about genetic and non-genetic biomarkers, vascular imaging, animal models and biocomputing, not only in one but all vascular diabetes complications on hand. Below we summarize SUMMIT's principle findings and Foreground generated during the last 6-years, for each of the SUMMIT phenotypes/indications. But let us first start with our achievements in **Predictive modelling,** which results represent several diabetic complications simultaneously

SUMMIT has achieved several goals during the six years of the SUMMIT project. From a computational point of view, several tools for the multivariate analysis and selection of Single Nucleotide Polymorphisms (SNPs) data were defined, validated, published and thus made available to the scientific community. These tools are called i) Hierarchical Naïve Bayes (HNB; Malovini et al 2012), ii) Binary Outcome Stochastic Search (BOSS; Russu et al 2012), iii) Bag of Naïve Bayes (BoNB; Sambo et al 2012) and iv) Algorithm based on a BivAriate CUmulative Statistic (ABACUS; Di Camillo et al 2014) and they are publicly available. A specific pipeline of analysis to be applied to SUMMIT data was defined based on part of these tools. UNIPV and UNIPD also collaborated to the definition of an *in silico* model for the progression of diabetes complications based on the analysis of public datasets, which has been recently published (Marini et al 2015) The described method was defined and validated on the publicly available Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) study cohorts publicly available through the NIH dbGaP repository.

Progress per indication:

Diabetic Kidney Disease (DKD)

SUMMIT represents the largest collection of **GWA data** available for diabetic complications and the only study of DKD extremes with whole exome sequencing data. Within the DKD genetics studies SUMMIT included a total of 5,908 DKD cases and 4,965 DKD-free controls from subjects with either T1D or T2D. A total of 7 binary DKD phenotypes and one continuous trait were analysed, a number of variants from each phenotype for replication selected for replication in suitable cohorts. At the end a promising locus from the microalbuminuria phenotype in subjects with T2D was identified that may also represent an eQTL for Gamma-aminobutyric acid type B receptor 1 (GABR1*)*. It was also demonstrated that the UMOD variant association with CKD extends to diabetic kidney disease. Other than supportive genetic data for UMOD, SUMMIT has however not been able so far to demonstrate unequivocal causality for the biomarkers observed to be associated with DKD, nor did the project identify any genome-wide signal significant for DKD in subjects with T1D.

SUMMIT … ***AND WHAT WE ACHIEVED:***

DIABETIC KIDNEY DISEASE IN T1D AND T2D

SUMMIT PHENOTYPE DEFINITIONS:

- *Harmonization of phenotype definitions and samples descriptions for a range of cohorts with different procedures in order to make optimal use of the unique sample and data material.*

SUMMIT DKD GENETICS:

- *Ground breaking GWAS approaches in T1D and T2D (5.908 DKD cases, 4.965 DKD free control) and one of the first ever exome sequencing efforts in DKD extremes to set the stage for gathering information on rare, coding variants not detectable using GWAS or candidate gene approaches – in total 7 binary phenotypes and one continues trait were analysed;*
- *Identified after replication loci with genome-wide significance from the microalbuminuria and eGFR phenotypes in T2D;*
- *Significant integration effort with the JDRF/DNCRI GWAS datasets in progress.*

SUMMIT DKD CIRCULATING BIOMARKERS:

- *Comprehensive biomarker discovery and validation approach including metabolomics, lipidomics and protein analyses and, pilot studies for miRNAs – more than total 980 biomarkers measured;*
- *Developed, validated and filed for patent a panel of 14 biomarkers that improve prediction of rapid progression of eGFR decline in T2D over and above clinical measures;*
- *Demonstrated and validated an inverse relationship between serum UMOD and risk of DKD in T2D, and identified and validated the combination of serum Beta 2-microglobulin and serum Kidney injury molecule 1 as predictive of declining eGFR in T2D.*

SUMMIT DKD ANIMAL MODELS:

- *Glomerular expression profile database generated both for T1D and T2D mouse models and at different time points after diabetes onset;*
- *Systematic characterization of 19 existing and new animal models highlighting that no single model can mimic the full spectrum of disease but that a range of models can mimic different aspects of disease;*
- *Two rat models, the ZSF and the BBDR.CG-lepr.cp (patent filed, license agreement under negotiation) were identified to present significant advantages compared to previously described models and exhibit CKD. On top of this, models were validated in intervention studies with standard of care compounds.*

In the SUMMIT **non-genetic biomarker discovery studies** 989 biomarkers were measured in total across a range of platforms, including a set of pre-specified biomarkers by ELISA (at UKE lab, Hamburg) and luminex technology (Myriad RBM), a panel of metabolites, peptides and proteins using mass spectroscopy (WellChild lab) and lipidomic profiles using the Eli-Lilly in-house platform. As published in Kidney International, in one of these studies, the rapid progressor discovery study, two panels of biomarkers - one including 14 and the other 35 biomarkers – were identified that maximise prediction of decline in eGFR among individuals with Chronic Kidney Disease stage 3 in addition to clinical covariates as judged by increases in AUROC and difference in test log likelihood. SUMMIT also showed that a significant improvement in prediction could be achieved by using a more extensive set of clinical covariates including a weighted average of prior measures of eGFR. For validation of the two biomarker panels, samples from Go-DARTS and the Scanian Diabetes Registry (SDR) were used. A

manuscript detailing these validation findings is in preparation. The DKD discovery and validation sample- and data-sets were in addition used to examine the prediction contained within a combination of a small number of biomarkers (<5) and, also, whether good prediction can be attained in a biomarker panel restricted to a single platform. This work is also being prepared for publication.

Addressing the lack of **animal models for DKD** two SUMMIT rat models, the ZSF and the BBDR.CG-lepr.cp were identified to present significant improvements compared to previously described models, as well as exhibit CKD. Both models were extensively characterized during the course of the project, including intervention studies with standard of care that were only enabled at a late stage of the project by a successful ENSO proposal being awarded to further develop the outstanding Foreground generated by the IMI project. A glomerular expression profile database was generated for both T1D and T2D mouse models and at different time points after diabetes onset.

Cardiovascular disease (CVD)

One of SUMMIT's key aims was to develop a novel, **non-invasive method to assess atherosclerotic plaque structure and vulnerability**. The project succeeded with this ambitious task by bringing forward a method (algorithm) that in principle is based on centre frequency shift (CFS) of the ultrasound radio frequency data as obtained from carotid plaques compared to a reference phantom. The so-called ultra-sound based plaques structure analysis (UPSA) was evaluated both *ex vivo*, on 157 sections from 18 plaques, and *in vivo*, in 39 patients one day prior to carotid plaque removal, and subsequent correlation of the data with histology. The CFS correlated with a plaque vulnerability index based on histological areas stained for lipids, macrophages, haemorrhage, smooth muscle cells and collagen (r=-0.726, P=0.000012). In conclusion our method is the first to characterize atherosclerotic plaque components that affect plaque vulnerability using CFS. This new technology has the potential to considerably improve preventive strategies, risk stratification and monitoring of interventions – and therefore plainly addresses "THE" SUMMIT overall goal - in the future. The UPSA was further validated through usage in the frame of the SUMMIT macrovascular study (see below) and found recently its way into the study programme of two additional Swedish cohort studies. A patent has been filed.

Most importantly, SUMMIT also completed its state of the art **prospective, multi-centre macrovascular study**, which investigates the ability of vascular imaging, functional tests and novel biomarkers to predict (1) new cardiovascular events or death (primary endpoint), and (2) progression of carotid disease (secondary endpoint) during a 36 months follow up period in subjects with or without T2D. The study included 458 subjects with T2D and CVD (myocardial infarction, stroke or lower extremity arterial disease), 527 subjects with T2D but no clinically manifest CVD and 515 non-T2D subjects with or without CVD recruited at the university hospitals in Malmö, Dundee, Exeter and Pisa.

… AND WHAT WE ACHIEVED:

CARDIOVASCULAR DISEASE IN T1D AND T2D

SUMMIIT CVD IMAGING:

- *Developed the novel non-invasive Ultrasound Plaque Structure Analysis (UPSA) technique that can determine plaque structure and identify high-risk plaques with specificity and sensitivity equal to intravascular ultrasound, MRI and CT/PET without having the costs and safety issues associated;*
- *Completed a prospective multi-centre study investigating the ability of vascular imaging, functional tests and novel biomarkers to predict new CVD events or death and progression of carotid disease (baseline and 36 months follow-up): one of the best characterized macrovascular studies up to date showing relevant differences in risk prediction for MACE between T2D and non-T2D subjects;*
- *Completed a whole body CVD magnetic resonance imaging study (158 study participants) suggesting that CVD events may occur at a lower atheroma burden in diabetes.*

SUMMIT CVD CIRCULATING BIOMARKERS:

- *Comprehensive biomarker discovery and validation approach including metabolomics, lipidomics and protein analyses and, pilot studies for miRNAs; - more than 950 biomarkers measured;*
- *Identified (in SUMMIT historical sample collection) several biomarkers - Apolipoprotein CIII, Interleukin-6, Interleukin-15, high sensitivity Troponin T, N-terminal prohormone B-type natriuretic peptide - associated with CVD and with N-terminal prohormone B-type natriuretic peptide and Apolipoprotein CIII in particular providing improved prediction of CVD over and above clinical covariates;*
- *Explored the associations of specific immune based biomarkers with incident CVD and identified several potentially useful biomarkers involved in atheroma;*
- *Identified (in SUMMIT new multi-centre study samples) an association of matrix metalloproteinases-7 and -12 (MMP-7, MMP-12) independent of Framingham Risk Score factors and most prominent in subjects with T2D supporting their role in plaque development and suggesting their usefulness as biomarker for vascular complications in T2D.*

SUMMIT CVD GENETICS:

- *Considerably expanded GWAS efforts in T2D: 10.012 cases, 14.245 controls;*
- *Identified after replication three known CAD SNPs that were also associated in CAD in T2D;*
- *Demonstrated that the genetic basis of predisposition to diabetes complications is even more complex than anticipated.*

SUMMIT CVD ANIMAL MODELS:

- *Systematic characterization of 19 existing models and 4 new animal models;*
- *Two mouse models, the GSK.ApoE and the IGF-II/LDLR-/-ApoB100/100 and one rat model, the BBDR.CG-lepr.cp (patent filed, license agreement under negotiation), were identified exhibiting characteristics of CVD and to present significant advantages compared to previously described models: intervention studies with standard of care compounds;*
- *Demonstrated the role of the calcium/calcineurin transcription Factor NFAT (Nuclear Factor of Activated T-cells) in the development of macrovascular complications and endothelial dysfunction.*

Baseline measurements included atherosclerotic burden (common carotid artery (CCA) and carotid bulb intima media thickness (IMT), total plaque area in the carotid bulb and ankle-brachial pressure index (ABPI), atherosclerotic plaque structure (UPSA), gray scale median (GSM), percentage echogenic and echolucent plaques, arterial stiffness as assessed by the pulse wave velocity (PWV) and endothelial function measured by Endo-PAT technology and expressed as the Reactive Hyperemia Index (RHI). Incident clinical events were monitored during a 3-year follow up investigation through patient interviews and/or hospital records. Follow-up data analysis will be completed in the first months of 2016 and the partners involved have agreed to do future follow-ups (5 and 10 years) of these valuable cohorts, to further increase the scientific value of the database. A number of macrovascular changes associated with diabetes was detected including aggravated atherosclerosis, increased arterial stiffness and endothelial dysfunction. Measures of atherosclerotic burden were found to be associated with clinically manifest CVD in T2D. In addition, vascular changes that are not directly related to known metabolic risk factors were determined to be important in atherosclerosis and CVD in T2D. A better understanding of the mechanisms involved is crucial for enabling better identification of CV risk in diabetes.
Like for other indications, SUMMIT has also undertaken a series of targeted studies looking at **pre-specified biomarkers or pathways** to identify new biomarkers for use in predicting cardiovascular diabetic complications. In total over 950 biomarkers were measured in the CVD discovery study across a range of platforms including a set of pre-specified biomarkers by ELISA (at UKE lab, Hamburg), luminex technology (Myriad RBM) and a series of high dimensional platforms including metabolomics (Metabolon) and lipidomics (ZORA).

Amongst others, a **panel of 92 novel and established biomarkers was analysed in new SUMMIT baseline samples** from the SUMMIT macrovascular study, using proximity extension assay with the goal to determine the association between vascular changes in T2D and a large number of biomarkers using the OLINK CVD multiplex platform (http://www.olink.com). CVD study patients were characterized by elevated plasma levels of MMP-7 and -12. This association was independent of the factors included in the Framingham Risk Score and was most prominent in subjects with T2D. Plasma MMP-12 was higher in subjects with T2D, increased with age and impaired renal function and was independently associated with atherosclerotic burden (as assessed by carotid intima-media thickness and ankle-brachial pressure index), arterial stiffness and plaque inflammation. These findings show that plasma level of MMP-12 is elevated in T2D and associated with more severe atherosclerosis and clinical manifestations of CVD. These observations also provide clinical support to previous experimental studies demonstrating an important role for MMP-12 in plaque development and suggest that MMP-12 represents a potential biomarker of vascular complications in T2D.

As a result a from the ELISA measurements of **pre-specified protein biomarkers** in samples from the SUMMIT historical cohort (see above) a panel of 6 biomarkers – Apolipoprotein CIII (ApoCIII), Interleukin-6, Interleukin-15, high sensitivity Troponin T, N-terminal prohormone B-type natriuretic peptide (NTProBNP) and soluble receptor for advanced glycation end products (sRAGE) – was identified as best predicting incident CVD and improved prediction of CVD over and above clinical covariates based on improvement of AUROC and difference in test log likelihood. Furthermore, by inclusion of some diabetic specific clinical covariates, such as diabetes duration, HbA1c and eGFR, prediction improved beyond that attained by use of the standard Framingham Risk Score. These data were reported in Diabetologia. Validation studies of the panels showed that the most useful

predictive information above clinical covariates is found using the combination of NTProBNP and ApoCIII and a manuscript summarizing the outcomes from the SUMMIT CVD protein biomarker study is currently in preparation.

Interesting initial associations of metabolites with CVD were also obtained from the metabolomics study but these were not replicated in the validation studies in the CARDS and FINRISK cohorts.

A series of SUMMIT experiments was implemented – mostly in the format of single partner activities - to generate new potential biomarkers from the known biology of complications. Based upon data from the Malmo Diet and Cancer study, exploring associations of specific **immune based biomarkers** with incident CVD, several potentially useful biomarkers and pathways involved in atheroma could be identified by SUMMIT researchers. Another project initially triggered by the known biology of complications was an approach to explore the **hexosamine flux pathway** as a potential source of biomarkers for diabetes complications. This work has identified biomarkers of diabetes, which may also be related to complications. Last but not least, a **non-invasive biomarker for *in vivo* platelet activation** was validated and **a novel assay for urinary 11-dehydro Thromboxane B_2** has been developed. The assay was used in studies on responsiveness to antiplatelet drugs and personalized regiments in elderly diabetic patients showing age- and BMI-related changes in the pharmacodynamics and pharmacokinetics of different antithrombotic drugs and differences in responsiveness to aspirin between people with T1D and T2D. This biomarker has been studied by using the novel assay as predictor of macrovascular complications in the ongoing ASCEND trial, on 8.000 urinary samples from T2D patients.

Within the **genetics studies** a total of 10,012 CAD cases and 14,245 CAD free controls from subjects with T2D were included. A number of promising variants from each phenotype were selected for replication and suitable replication cohorts were identified. SUMMIT identified three known CAD SNPs that were also associated with CAD in subjects with T2D.

A number of existing and newly created **animal models** have been characterized during the course of the project are potentially interesting in the CVD context. The GK.ApoE mouse, the diabetic Akita mouse, the SUR1xIGFII and SUR1xLDLR KO mice, the Apo db/db mouse, the IGF-II LDLR-/- ApoB 100/100 mouse, CUGBP-1 LDLR KO/ApoB100/100 transgenic mouse, the ANRIL KO LDLR KO/ApoB100/100 mouse, the BBDR.cg-lepr.cp rat.

The ENSO drug intervention studies were performed on 3 of these models, the GK.ApoE mouse, the IGF-II LDLR-/- and the BBDR.cg-lepr.cp rat; and the analyses are being completed. Resources and interest are in place to run additional measurements on samples from these studies, such as metabolomics on the rat model and RNAseq on rosuvastatin-treated IGFIIxLDLR KO/ApoB100/100 mice.

The diabetic IGFIIxLDLR KO/ApoB100/100 mice developed at the SUMMIT partner in Kuopio (re-derived and deposited at Taconic for sustainability) were earlier on studied for their cardiac (Heinonen et al 2011) and ocular phenotype (Kinnunen et al 2013). Since the latest report, the response of this new mouse model to pathological cardiac hypertrophy has also been studied and the manuscript is currently being prepared.

SUMMIT successfully developed and characterized two new diabetic mouse strains (SUR1xIGFII and SUR1xLDLR KO) with altered insulin metabolism. Analyses of atherosclerosis burden are ongoing and

a manuscript is planned. The SUR1 model was also used for studies on epigenetic modifications in the kidney, summarized on a paper in press in Kidney International (De Marinis et al, in press).

In addition, CUGBP-1 transgenic and ANRIL KO mice on LDLR KO/ApoB100/100 mice were generated based on SUMMIT GWAS findings indicating a CVD related phenotype. With the GWAS hits being available only in a fairly late stage in the project and model generation taking its time, the cardiovascular characterization of these models will be continued post-SUMMIT (metabolic characterization though completed). Interesting hyperglycemia effects on gene expression in relation to vascular tissues in mice as well as human tissues were described in Daskoulidou et al (2015) and Hien et al (in press).

Diabetic Retinopathy (DR) & Lower Extremity Arterial Disease (LEAD)

In clinical trials the gold standard definition of diabetic retinopathy (DR) progression and regression is a three- or two-step change on the ETDRS grading scale (11 assignable levels of increasing severity for retinopathy, from none to advanced proliferative diabetic retinopathy). To detect changes of these magnitudes with potential interventions, particularly in the early stages of DR, results in clinical trials of long duration, potentially delaying the instigation of new, effective treatments. Technologies or approaches that may aid both the early identification of individuals at risk of DR and shorten the length of clinical trials are continually being sought. One such avenue is **optical coherence tomography (OCT)** that non-invasively images the retina for the quantitative measurement of macular thickness. The aim of the **SUMMIT study** was to examine whether macular thickness measurements by OCT can predict DR.

Participants with diabetes mellitus and either no DR up to mild DR were recruited across three centres (Exeter, Lund and Dundee). Macular thickness and clinical assessments were performed at baseline, and then repeated ~ 3 years later. Retinal grading was performed from 2 field retinal photography (45^{o} nasal and macular view) using a modified Scottish Diabetes Retinopathy Grading scheme. Macular thickness was measured by OCT (Cirrus HD-OCT or Topcon-1000 OCT). Progression was defined as a 2 step + change on the DR grading scheme in one eye. Non-progression was defined as no increase in DR over a minimum of 30 months follow-up. The OCT data from the corresponding eye entered the analysis. There was no difference in the thickness of any of the macular regions when comparing progression group (n = 46) to the non-progression group (n=525). Subgroup analysis on participants with no DR at baseline demonstrated a reduced fovea thickness at baseline in the group that developed DR (2 step or more increase in DR) (n =29) compared to individuals that remained DR free (n = 313) (mean: 258 (23) vs. 268 (SD: 22) respectively, p = 0.014 independent t-test). The association between baseline fovea thickness and DR development was independent of baseline HbA1c (logistic regression, fovea thickness odds ratio: 0.978 (0.960, 0.996) (p = 0.016), HbA1c: 1.039 (1.012, 1.067) (p = 0.005).

However, the association of baseline fovea thickness with incident DR was not sufficient to significantly improve prediction of incident DR as assessed by receiver operated curves (area = 0.605 (std error: 0.051, p = 0.061).

In summary, this study has demonstrated that the measurement of net macular thickness by optical coherence tomography lacks the sensitivity and specificity to be a useful diagnostic test to predict

DR. However, it does provide novel, longitudinal physiological data on the relationship between macular thickness and the earliest stages of DR that with further interrogation of the data will increase our understanding of the development and progression of DR.

For both phenotypes, DR and LEAD, we were restricted in the **non-genetic biomarker search** by limited data for the phenotype within SUMMIT but were able to carry out sub-analyses of the CVD data. For DR we identified a biomarker for progression of disease and a biomarker for the incidence of LEAD.

Within the **genetics studies** we included a total of 2,621 LEAD cases and 14,579 LEAD free controls from subjects with either T1D or T2D and 5,422 DR cases and 4,302 DR free controls from subjects with either T1D or T2D. We did not identify any genome-wide significant for LEAD or DR in subjects with either T1D or T2D. However, continued efforts on this topic are expected to increase both sample size and statistical strength of the data.

No **animal model** could so far be identified that better replicated DR than the already available standard models.

SUMMIT ... ***AND WHAT WE ACHIEVED:***

DIABETIC RETINOPATHY (DR) /

LOWER EXTREMITY ATERIAL DISEASE (LEAD) in T2D

SUMMIIT DR IMAGING:

- ***Generated the largest dataset of Optical Coherence Tomography (OCT) measurements with longitudinal follow-up to date (multi-centre study, 1.3200 study subjects) investigating whether macular thickness measurements by OCT can predict diabetic retinopathy and comparing OCT against Fluorescein Angiography (FA), the clinical standard tool of the retinal vasculature;***
- ***Developed a method to standardise OCT measurements in multi-centre trials and clinical practice.***

SUMMIT DR and LEAD CIRCULATING BIOMARKERS:

- ***Discovered (SUMMIT historical sample collection, limited numbers for DR) a restricted number of biomarker candidates for each, DR and LEAD;***
- ***Additional DR biomarker candidates expected from the ongoing analysis of samples from the new DR multi-centre study.***

SUMMIT DR and LEAD GENETICS:

- ***Performed DR GWAS analysis in 5.422 DR cases and 4.302 DR-free controls;***
- ***Performed LEAD GWAS analysis in 2.621 LEAD cases and 14.579 LEAD-free controls;***
- ***No signal with genome-wide significance identified so far.***

SUMMIT DR (no LEAD) ANIMAL MODELS:

- ***Systematic characterization of 19 existing models and 4 new animal models;***
- ***No improved models above the current standard identified / generated so far, analysis of one mouse and one rat model ongoing.***

SUMMIT has achieved its goals. Although there is still some way to go, to fully establish our outcomes in pre-clinical and clinical research schemes, we can say without exaggerating that after SUMMIT we can better predict the major diabetes complications as investigated in SUMMIT, here to name are at the foremost DKD and CVD and our biomarker panels and predictive *in silico* tools. The advantages the novel UPSA technology for non-invasive plaque structure assessment will bring for future clinical trials - but also the daily praxis – are obvious. The knowledge and models generated by our animal experts are most important for the pharmaceutical companies. The comprehensive characterization of our new models and of a good number of already existing models showed that each model, although most of them are defined by an early onset of diabetes, has its specific features when it comes to diabetes complications, an information most essential for pre-clinical research by the pharmaceutical industries but also the academic sector. With completion of the SUMMIT intervention studies, the four selected SUMMIT animal models will be amongst the best characterized model organisms for diabetes complication research in DKD and CVD and ready to use by the pharma industry and others. Our genetics efforts comprised by extensive GWAS and exome sequencing efforts identified several loci with genome-wide significance. Though we hoped for more, the stage is now set to make significant progress in understanding the genetic basis of predisposition to diabetic complications and underlying key pathogenetic mechanisms in the near future. Important will now be to take steps to efficiently develop the SUMMIT Foreground to the next level.

1.6. Potential impact and main dissemination activities and exploitation of results

SUMMIT has provided vital data on biomarkers for diabetes complications, which we would hope could be fully exploited in the future. To understand the **potential** it is worth considering the possible ways that these data may be used which include: i) improving prediction of outcome; ii) providing evidence of the pathogenic pathways involved in thus leading to potential novel drug target identification, and iii) novel techniques and models for potential development of new drugs.

***i) Improving prediction of risk*:**

Improving our ability to identify individuals at risk for a specific complication of diabetes means that the treatment options for that person become clearer and their chances of preventing or delaying the onset of the complication increase. Thus, the goal is to reduce the number of people developing the complication. As we continue to see increases in the prevalence of diabetes across the world this becomes more and more vital. Therefore, our work will aid to improve detection of CVD and DKD risk in T2D, including the importance of clinical covariates not currently included in the major risk scores.

SUMMIT represents one of the major efforts worldwide to identify genetic variants that influence risk of diabetic complications, and to combine these data with biomarker measures in the search for causal surrogate measures of DKD progression. Though the findings from SUMMIT alone have not yet been translated into novel exploitable clinical opportunities, ongoing efforts to combine SUMMIT data with those derived from the JDRF-DNCRI (for DKD), from CARDIOGRAM (for CAD), and from the global meta-analysis efforts for DR and LEAD, offer improved sample size and additional opportunities for biological inference that will underpin future efforts to validate novel therapeutic targets, and to define novel causal biomarkers. These efforts may be carried forward through the

AMP-T2DGENES effort in the States (which will be able to access published summary level data from SUMMIT), and collaborations within future IMI diabetes projects.

Biomarkers also have a slightly different role for complications where preventative treatments are less available. For DN the major steps for prevention relate to blood pressure management, good glycaemic control, and blockade of the angiotensin-renin axis. As such there is great interest in identifying drugs that will further delay progression of renal decline and prevent or at least delay the onset of end stage renal disease. This is an area of great interest to the biopharmaceutical industry. One of the elements that has caused difficulties in developing such new therapies has been the problems relating to identifying individuals at high risk of events for inclusion in clinical trials, as these trials are powered by events and, with low number of incident cases, trials have to recruit more people and run longer, both of which increase the cost of the trial. We have shown how the improvement in prediction seen by use of the panels we defined translates to proportion of people at risk for progression in a study, which could lead to fewer people being followed for less time to attain the same degree of power for a study that did not make use of enrichment. We have also shown the potential utility of a smaller combination of biomarkers measured only on a single platform which may be logistically more useful for both clinical trial risk assessment but for patient assessment for clinical purposes.

The other area where the biomarker work could be exploited is to work with companies with an interest in developing and marketing assays, as the multiplexing of some of the biomarker combinations we have identified may be commercially viable. To this end we have applied for a patent centring on the DN biomarker work so that this work can be exploited financially if non-SUMMIT parties wish to develop it further.

ii) *Providing evidence of pathogenesis of complications*

Whilst prediction is greatly important, learning more about the pathogenesis of diabetic complications is also vital. Identification of biomarkers can lead to the identification of important pathways that may contribute to our understanding of the pathogenesis of the disease. When the biomarker pathways have a causal relationship with the complication, this can then provide vital information as to potential novel drug targets for treatment, potentially leading to new and important treatment options for patients. SUMMIT generated biomarker and genetic data for complications, making us better able to determine when a biomarker might be causally related to a complication and thus whether new pathways should be investigated. SUMMIT experimental work also identified novel pathways promoting diabetes complications (i.e. signalling via the Ca/calcineurin-dependent transcription factor NFAT, involvement of ORAI channels in kidney tubular function, hyperglycemia-driven modulation of smooth muscle phenotype in the arterial wall).

iii) *Novel techniques and models for potential development of new drugs*

Development of reliable surrogate endpoints for cardiovascular protection represents a major challenge in the clinical development of new drugs for cardiovascular event reduction. Moreover, this is a particular challenge in diabetes since most drugs have failed to demonstrate reduction in cardiovascular events in spite of good metabolic effects until recently, with the SGLT2 inhibitor Empaglifocin being the exception from the rule (see EMPA-REG-OUTCOME study). SUMMIT has performed a comprehensive study of the ability of different vascular imaging techniques, functional methods and novel biomarkers to predict progression of vascular disease and development of clinical events. These studies have identified combinations of non-invasive vascular imaging techniques and

novel biomarkers that predict progression of disease but also have shown that these methods provide less reliable results in subjects with diabetes. This knowledge will be of considerable importance for guidance of the design of future phase I/II studies.

Optical coherence tomography (OCT) devices that measure macular thickness are routinely used in clinical practice and research. However, the interpretation of the measurements is complicated by the fact that the software used for the automated measurement of macular thickness varies between different types of OCT devices, resulting in systematic differences. Work within the SUMMIT Diabetic Retinopathy study has developed a potential method to standardised OCT measurements in multi-centre trials and clinical practice. This work will make a valuable contribution to the discussion in this area. Preliminary data analysis of the DR study has demonstrated that the measurement of net macular thickness by OCT lacks the sensitivity and specificity to be a reliable diagnostic test to predict DR. However, it does provide novel, longitudinal physiological data on the relationship between macular thickness and the earliest stages of DR that with further interrogation of the data will increase our understanding of the development and progression of DR.

We have invested efforts to make sure the animal models developed by the consortium, which are improved models for diabetic complications and therefore invaluable for research, are made available to the scientific community and pharmaceutical industry, via rederivation and storage of embryos at commercial vendors (either via MTAs or licensing). Results from SUMMIT's animal model work have been published during the course of the project, and we anticipate additional publications to emerge from data generated by the consortium even after the end of SUMMIT. Protocols are made available via these publications.

The definition of robust multivariate predictive models represents a topic of great interest for the scientific community, especially in the context of personalized medicine. As an example, the identification of predictive models based on the combination of genetic and clinical information could allow stratifying patients based on their characteristics and thus assign them to specific risk categories. Preventive treatments based on these decisional algorithms will allow a significant reduction of the probability of developing diabetes complications, with a great benefit for the patients' health and quality of life, allowing also for a significant reduction in terms of treatment costs. The introduction in the clinical practice of validated risk stratification schemas and *in silico* models for the simulation of the diabetes complications could allow simplifying the decisional process of clinicians by interpreting the individual level characteristics of the patients, thus offering the opportunity for a further step toward personalized medicine. In this context, one of the main advantages offered by the personalized medicine is represented by the possibility to define personalized therapeutic strategies, which allow maximizing the efficacy of the treatment while minimizing the probability of side effects.

1.7. Lessons learned and further opportunities for research

Collaborating in a PPP – the SUMMIT experience:

"The benefit of a PPP is larger than the energy invested to make the collaboration work"

We cannot deny that there were cultural differences between academic and industry partners, when we entered into this collaboration, yet we have grown together and taught each other valuable lessons. There have been many specific examples of the added benefit of a PPP approach for the project and we have outlined some examples here.

1 + 1 > 2: Both academia and industry partners have gained access to competences and expertise and indirectly to data and other resources that they would not otherwise have had access to in the academic/public domain. The benefits have been gained from both increase in the critical mass of data available for investigation and the intellectual momentum gained by including more individuals with diverse knowledge from both industry and academic settings.

Sharing a view: In SUMMIT we had both academic and EFPIA leads ensuring that both perspectives were fully represented in all our planning. We also benefited from a wide range of knowledge and expertise due to the pre-competitive nature of the IMI-JU arrangement.

Exchange of knowledge: Direct contact between academic and non-academic partners (i.e. pharmaceutical companies) boosted the activities by providing a fresh and alternative view on many aspects of the research. In a near future, these collaborations could helpful in translating research findings into globally available products. In this context, it was extremely useful that young academic researchers e.g. were allowed to spend part of their PhD period at AstraZeneca in Gothenburg, Sweden or at other academic institutions. Useful collaborations were established also between clinical and data modelling partners, opening up for further collaborations.

Biomarker selection: One of the key decisions we had in WP2 was to identify the existing putative biomarkers to include for any given phenotype. Whilst some were identified as important by all we also had the advantage of having investigators championing specific biomarkers and platforms, which proved invaluable to the project. For instance experience at Roche and Sanofi-Aventis helped identify the best company that could undertake high quality biomarker measurements for SUMMIT, which in both the CVD and DKD studies have emerged as some of the most predictive biomarkers assessed.

Study design: Academia and industry came into the project from partially different backgrounds of experience and knowledge, something that could be used to our advantage. A clear example of this was when we came to design the rapid progressor discovery study, as the role of biomarkers for DKD is currently of great interest to EFPIA in order to improve the efficiency of clinical trials. Here we were able to work together closely to come up with the phenotype employed and also in how best to present the results with the use of positive predictive value plots rather than simply relying on differences in area under the receiver operator characteristic curve (AUROC) to better demonstrate how a set of biomarkers could be used to enrich a clinical trial.

Funding for subcontracting within SUMMIT: Due to the limitations on reimbursement it was difficult for academic partners to sub-contract biomarker work in SUMMIT. Not all biomarker studies could be undertaken in-house, neither at the participating academic institutes nor at the industry partners. We worked closely with all our EFPIA partners to make the best use of IMI funds for sub-contracting. We are pleased to see that IMI-2 funding rules provide a more liberal reimbursement of costs.

Opportunities for analyses: In addition to providing funds for sub-contracting academic partners received valuable in-kind support from EFPIA partners, which ranged from providing chips for the

GWAS studies and assay kits to labs for biomarkers measured in the CVD and DKD studies to in-house measuring DKD samples on shotgun - and targeted lipidomics platforms.

Statistical analysis and modelling skills: SUMMIT has been fortunate to have outstanding biostatisticians and machine-learning experts from both academia and EFPIA in the team. Collaborations with academic partners allowed exchanging key experience in several research contexts thus improving the knowledge about clinical topics that could have been difficult to deepen otherwise.

Animal models: The **Public Private Partnership (PPP)** collaboration has been extremely fruitful for the animal model work, not only due to complementary competences and expertise, but also by allowing for a larger battery of resources to be applied effectively to solve a specific task. EFPIA partners have a large experience in and extensive information on drug intervention trials, which has been extremely useful for our ENSO project. Several spin-off and side projects have emerged in the animal model work in the SUMMIT PPP and we can easily envision continued collaboration and communication between most WP4 partners even beyond the termination of SUMMIT.

When considering further PPP work we would identify a few areas where we would have benefitted from even greater links. In order to maintain and maximise interactions and support between academic and industry partners we recommend:

Large-scale collaborations – and this is even more true for large PPPs – demand joined responsibility and extensive efforts regarding communication and we would like to see an further improved communication through

- Even more regular teleconferences and face-to-face meetings;
- More high level as well as focussed discussion groups like e.g. the indication workshops initiated half way throughout the project, meeting up regularly;
- Clearly defining, from the start, what expectations the partners have of the collaboration and which possible inputs can be expected;
- More active involvement in project governance on all levels, e.g. tasks, workshops and the overall project: raise critical questions, contribute to solutions, take responsibility.

Improved availability of

- Data: It is important to make data both public and internally generated easily obtainable where sharing is appropriate. Pharma colleagues should be encouraged to make more of their wealth of Background data available to the joint enterprise (e.g. clinical trial data). We were surprised not to be able to access as much of the existing bioinformatics data that the EFPIA partners have generated over the years. It was unclear whether this was a case of the expertise being outside the remit of the investigators engaged in SUMMIT or internal procedures making this data hard to share. In addition, with SUMMIT analyses relying to a large extend on historical cohorts, the alignment of access allowances proved to be a larger task than anticipated, something that apparently many IMI projects encountered.
- Samples: Better access to EFPIA trials and samples would have facilitated the project because of them being more up to date, having more stringent phenotype definitions and having often more than one sample per study patient. These samples would have also helped to more easily

overcome the limitations experienced for the availability of validation samples. We feel that we were not able to make as much of this potential resource as we would have liked and providing greater sharing would have been helpful to the project by allowing us a wider range of samples for validation studies. However, although addressed early on with the IMI the value of such sample sets is still unclear and IMI-1 financial provisions did not consider any value for samples collected before the project's start.

- Expertise: Collaboration on analysis was more limited than we had hoped as sometimes it became separate teams working – though we also have some very good examples for real collaborations that may in many cases exceed SUMMIT - on projects rather than true collaboration and this may be an element of such collaborations where even more face-to-face meetings or chances to truly work together on analytical approaches would have been more beneficial. The scientific value contributed by different EFPIA companies was highly variable: there should be better ways of ensuring that "in kind" contributions are efficiently directed towards the scientific objectives of the project.

SUMMIT ***… LESSONS LEARNED FOR THE FUTURE:***

SOME CONSIDERATIONS, LOOKING BACK

- ***A call has to be realistic, the objective to achieve biomarker discovery, validation and translation of the newly discovered biomarkers in clinical setting was over ambitious;***
- ***The timing of ongoing and new calls needs to be improved: it is difficult to ask the right question in a new call before you have the full picture from the preceding ones, some IMI calls started too early;***
- ***Participation in a call is a joint responsibility and needs clear expectations from each partner;***

- ***Phenotype definitions are important, if we define a phenotype too broadly, e.g. all diabetes not being T1D is T2D, we lose, we have to improve in defining subtypes of T2D;***
- ***Samples have to reflect today's situation, in a setting of a continuously growing prevalence of diabetes it may make a difference if it was 3 or 10% at the time of collection or what intervention the patients were undergoing;***
- ***In addition to raising the sample and data availability from EFPIA, a more systematic approach will be required including stronger involvement of the health care system/providers;***
- ***Not all relevant questions may have been asked, future activities should take into account potential epigenetic effects of glucose;***
- ***Blood-born markers were investigated in depth, limitations in sample availability made us miss on urinary biomarkers, a gap to be closed in the future;***
- ***We added genomics to the diabetes complications landscape but still only few genetic panels exist that can be used for disease protection and progression, based on the solid basis established by SUMMIT numbers need to be further raised;***
- ***Retrospectively a more focused biomarker approach with more standardized samples and metabolomics analyses could be envisioned, technological progress may open up opportunities for innovative discovery opportunities for protein markers going beyond the measurement of candidates from the literature.***

Future research

SUMMIT has generated a lot of very useful and important data but as always there is more still to do. Some of the findings in SUMMIT have been surprising and have the potential to open up new directions in research. We are where we wanted to get and beyond but there are still open questions and opportunities:

The genetics work on diabetes complications will continue its search for genome-wide significant signals and many of the promising hits will be followed up. The SUMMIT-JDRF collaboration will extend some time over the final date of SUMMIT, to complete the joint analyses.

DKD – applications have been made for additional support via the recent IMI2 calls and proposals with strong SUMMIT participation have been or are in the race to be awarded funding, here to name are BEAT-DKD and RHAPSODY. These focus amongst others on the development of robust biomarkers through the integration of longitudinal clinical data from academic partners and from pharma. We are working with JDRF/DNCRI to extend genetic studies into DKD: these will provide mechanistic insights and causal inference to support biomarker discovery and validation efforts. Additional activity in this space will be possible via AMP-T2DGENES: though direct SUMMIT participation in this effort has not yet been supported, AMP will in any event benefit from access to the summary level data from SUMMIT made available at the time of publication. Future studies will also specifically address T1D.

There are opportunities to extend biomarker discovery using new imaging modalities, and through novel analyte measurements (e.g. protein arrays). Access to cellular models of disease and to genomic information from relevant tissues (e.g. kidneys) will support mechanistic and translational studies

The LEAD association data will be boosted by additional samples e.g. from the UK biobank.

Efforts to map genes influencing risk of DR will be dependent on large-scale collaborative efforts involving groups in Europe, North America and Asia.

Exploring biomarker work also in other settings may include assessment of biomarkers in T1D and in other ethnicities, and for DN also looking at earlier stages of renal decline.

An emphasis shift towards urinary biomarkers for DKD, as opposed to a main focus on plasma biomarkers, could be informative. Since SUMMIT started, several groups have developed urine biobanks. Unfortunately there were only few T2D urine samples with prospective data available at the time of the discovery phase in SUMMIT.

Putting biomarker panels into practice: While we have shown that our panels are reproducible in improving prediction for DN the real test is whether they can be used to enrich clinical trials. This will require EFPIA partners to utilise this information.

One of the key take-home messages from SUMMIT is that research will benefit from a more careful selection of animal models depending on the question to answer (as opposed to using animals based on traditions in the field). Validation of animal experiments at different facilities should be considered, given the extremely large phenotypic differences observed when the same animals were studied in different sites.

As indicated earlier in this report, the CVD clinical studies initiated in SUMMIT will organize additional follow-ups, for an extension and enrichment of the already valuable database.

The observations made in the macrovascular clinical study challenges some of the current concepts of cardiovascular complications to diabetes. First, the incidence of cardiovascular events was not higher in subjects with T2D than in those without T2D. This is, on the other hand, in line with other recent studies of a remarkable decline in cardiovascular mortality in subjects with T2D. Second, although baseline severity of carotid disease and degree of arterial stiffness predicted the risk for development of cardiovascular events and death in non-T2D subjects, it did not do so in subjects with T2D. Third, progression of carotid disease was associated with increased plasma levels of biomarkers reflecting repair processes while regression was associated with increased plasma levels of pro-inflammatory biomarkers. The latter observations question the current view of the pathogenic role of inflammation in atherosclerosis and in particular vascular complications to diabetes.

The original hypothesis of the diabetic retinopathy (DR) study was that a thicker macular would be associated with the development and progression of DR in its earliest stages. However, initial analysis of the data suggests that a thinner fovea thickness, possibly resulting from retinal neural loss, is associated with a greater risk of developing DR. As the retina is a neurovascular unit, further research is needed to differentiate the relationship between neuro- and vascular degeneration with DR and the relationship between structural and functional changes in the earliest stages of DR.

The six years of SUMMIT was not long enough to take a biomarker from development through validation by regulatory bodies such as the EMA or FDA though this remains the goal. However, as we have principally found evidence for combinations of biomarkers rather than a single biomarker this process may be quite complex.