1. Executive summary Reporting Period 3

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 (nephropathy or DN), 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.

1.2. Overall deliverables of the project

SUMMIT aims to identify novel 1. genetic markers and 2. soluble biomarkers, which can be used for prediction and monitoring the development of diabetic vascular complications, 3. to develop novel and improve existing imaging techniques for monitoring progression in atherosclerosis and retinopathy, 4. to develop novel animal models for micro- and macrovascular complications and 5. to develop novel *in silico* methods for predicting diabetic complications. The ultimate goal is to make these markers accepted by regulatory authorities and to disseminate the findings not only to the scientific community but also to lay people and patient organizations.

1.3. Summary of progress versus plan since last period

After 2 years of developing methods and collecting data, SUMMIT now moves on to harvest the fruits.
The discovery data, techniques and knowledge from the different parts of the project are integrated around each of the disease (sub-)indications (CVD, DN, DR, LEAD) to further efforts at biomarker identification. Combining all expertise for each of the indications within the consortium gives a multi-disciplinary view on the research questions. SUMMIT's scientific weight lies especially in the large, unique datasets for CVD and DN, though also DR data collection exceeds previous efforts.

GWAS and exome sequencing sample numbers were increased to improve the analytical strength and the laboratory work is now close to finishing. We completed analyses of ~2.5 thousand variants for a range of DN and CVD phenotypes and initiated analyses for DR and LEAD.

SUMMIT acquisition of lipidomic, metabolomic and putative biomarker data was accomplished and associations of these with CVD and DR in T2D were analysed. Clear progress was made in relation to autoimmune markers and CVD. SUMMIT participants jointly designed studies of biomarkers in rapid progression of renal function decline in T2D.

Further actions in DN included validation of a fully quantitative stable isotope dilution es-MSMS platform for candidate metabolites and a cross-sectional study of nephropathy biomarkers. A pilot on serum microRNA estimation to prognose progression of DN in T1D and T2D was initiated but later discontinued when commercial assays declared to be sufficient for small volumes were shown to be unreliable. New methods were developed for measuring hexosamine flux markers and thromboxane (TX)B_2, a major enzymatic metabolite of TXA_2 in low urinary samples.

In imaging techniques the most important progress has been the development and validation of a first version of the ultrasound/radiofrequency based virtual histology for non-invasive assessment of plaque structure. The method identifies so called vulnerable atherosclerotic plaques with a sensitivity that by far exceeds traditional ultrasound. The macrovascular baseline investigation already includes 85-90% of the patients to be recruited. A sub-study with whole body MRI angiography will render a gold standard for atherosclerosis severity and the validation of other imaging techniques. SUMMIT is completing a clinical study to examine whether pulse wave velocity can serve as surrogate marker for T2D CVD risk by comparing postprandial hyperglycemia and nearly normal postprandial glycemia.

During year 3 SUMMIT participants compiled reports on current and SUMMIT-developed animal models of diabetic complications, on the role of the immunological system in developing diabetic complications and on the development and use of a bisphosphonate-based MRI probe. We analysed functional stiffness parameter analysis in the ApoE db/db mouse model, studied the role of transcription factor NFAT in development of vascular complications of diabetes, described the first diabetic glomerular transcriptome and successfully measured vascular inflammation in mouse coronary arteries by PET measurements.

In silico modelling participants in SUMMIT have focused on setting up tools and pipelines for the multivariate analysis of SUMMIT data. We finalized and published three software tools for multivariate selection of genetic markers. By using external datasets a new *in silico* model of diabetes complications was developed, based on Dynamic Bayesian Networks, and the performance of predictive methods was assessed.

SUMMIT and IMIDIA consortia completed their Memorandum of Understanding and, together with the DIRECT consortium, created an information folder on the IMI Diabetes Platform. Key SUMMIT publications summarizing the discovery phase are in preparation.

1.4. Significant achievements since last report

SUMMIT completed the largest-ever GWAS for a number of major diabetes complications and generated almost all the data for the first-ever exome sequencing study of DN.

In a nested case-control study of 2300 incident CVD cases interesting novel associations between diabetic CVD and lipids, interleukins or metabolites could be shown and the strength of association of some of the putative biomarkers with CVD and DR was quantified.

SUMMIT developed and *ex vivo* validated a first version of the ultrasound/radiofrequency based virtual histology for non-invasive assessment of plaque structure, a methodology offering unique and cost-effective opportunities for monitoring of response to treatment in a clinical setting.

SUMMIT has generated 4 new mouse models and a unique rat strain, which replicate diabetic vascular complications and represent promising new models that can be used for pathogenetic studies as well as for the development and evaluation of new therapies.

The SUMMIT *in silico* modelling team published three software tools for genetic marker discovery and assessed the performance of these methods for prediction and classification of diabetes complications. A new *in silico* model of diabetes complications was implemented.

SUMMIT advanced discovery efforts to the point where it could launch into a consortium-wide, multi-disciplinary integration of data for each disease indication, with a special focus on CVD and DN.