Universidade de Lisboa

Faculdade de Farmácia

Scientific Advances in Diabetes:
The Impact of the Innovative Medicines Initiative

Maria de Fátima Martins de Brito

Dissertation supervised by Professor Maria Beatriz da Silva Lima and co-supervised by Professor Carla de Matos Torre

Master in Regulation and Evaluation of Medicines and Health Products

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Abstract

Innovative Medicines Initiative (IMI) is a public-private partnership between the European Community, represented by the European Commission, and the European Federation of Pharmaceutical Industries and Associations. This joint undertaking aims at accelerating the medicines development process and generating new scientific knowledge to promote the implementation of personalized medicine for priority diseases established by the World Health Organization. Currently, two IMI programmes have been undertaken, the first one (IMI1) was carried out from 2008 until 2013 and had a budget of €2 billion, and the second one (IMI2) was developed from 2014 up to 2020 and the budget committed was up to €3.276 billion.

Diabetes Mellitus is one of the World Health Organization's priority diseases under research by the IMI programmes, mainly due to the exponential increase of its global prevalence over the years. Between 1980 and 2014, this rate quadrupled from 4.7% to 8.7% in adults aged 18 years and older and the 20 years- World Health Organization's projections indicate that it could reach more than 20% of the population. Simultaneously, the mortality rate and the healthcare costs associated with diabetes have been increasing. Regarding mortality, diabetes was the seventh leading cause of death in 2016. In terms of economic impact, currently, diabetes and its related complications, such as cardiovascular diseases, diabetic kidney disease and diabetic retinopathy, represent a significant economic burden on the healthcare systems. Worldwide, the estimated annual costs of diabetes have increased from $232 billion to $760 billion, between 2007 and 2019.

In the Diabetes field, the main aim of IMI1 and IMI2 programmes is to shorten the prevalence of this disease, through the development of knowledge and methods that enable the implementation of personalized treatment for diabetic patients. Up to October of 2019, thirteen projects were funded by IMI for Diabetes & Metabolic disorders, more precisely SUMMIT, IMIDIA, DIRECT, StemBANCC, EMIF, EBiSC, INNODIA, RHAPSODY, BEAT-DKD, LITMUS, Hypo-RESOLVE, IM2PACT, and CARDIATEAM. Of these, INNODIA aimed at type 1 diabetes, DIRECT, EMIF and RHAPSODY were focused on type 2 diabetes, SUMMIT, BEAT-DKD, LITMUS, Hypo-RESOLVE and CARDIATEAM were related to complications of diabetes, and the remaining projects, namely StemBANCC, EBiSC, IMIDIA and IMI2PACT, were directed to scientific research.

In general, a total of €447 249 438 was spent by IMI in the area of Diabetes. However, there is a substantial lack of integration of achievements between the different projects, which prompted the development of this dissertation: "Scientific Advances in Diabetes: The Impact of the Innovative Medicines Initiative".

This dissertation' objectives were to collect the data of the funded-projects and integrate them into the following research axes: 1) target and biomarker identification, 2) innovative clinical trials paradigms, 3) innovative medicines, and 4) patient-tailored adherence programmes.

The research methodology applied was a literature review and the data sources used were the official project's websites, contacts with the project's coordinators and co-coordinator and the CORDIS database. From the 662 citations identified, 185 were included.

Through the integration of the data collected from IMI-funded projects, it was verified that for Target and Biomarker identification, the main achievements were in order to 1) identify and validate biological markers, tools and assays, 2) identify determinants of inter-individual variability, 3) understand the molecular mechanisms underlying the disease, 4) develop a platform of pre-clinical assays, and 5) develop systems' models. Therefore, several biomarkers, tools, inter-individual variability factors, including genetic markers, and relevant pathways were proposed for type 1 diabetes by INNODIA, for type 2 diabetes by SUMMIT, IMIDIA, DIRECT and EMIF, for pancreatic β-cells by IMIDIA and RHAPSODY, for diabetic kidney disease by SUMMIT and BEAT-DKD, and for cardiovascular diseases and diabetic retinopathy by SUMMIT. Moreover, new tools and assays to improve research field were developed by StemBANCC, EBiSC and IMIDIA. Also, two models for patients' stratification were proposed, one related to glycaemic control in patients with type 1 diabetes established by INNODIA, and another corresponding to the identification of subtypes of diabetes patients developed by BEAT-DKD/RHAPSODY.

Regarding the clinical trials, the data collected SUMMIT, DIRECT and BEAT-DKD corresponds to new clinical endpoints and trial designs to accurately reflect the characteristics of the diabetic subpopulation under test.

In terms of innovative medicines, information retrieved by SUMMIT, IMIDIA, DIRECT, StemBANNC, EMIF, INNODIA and BEAT-DKD consists on the identification of new therapeutic targets and the development of agents with the purpose of treatment and prevent diabetes and its related complications. Furthermore, a new approach for the large-scale production of human pluripotent stem cells was proposed by StemBANCC.

Concerning the maximization of beneficial health patient-centred outcomes, two novel predictive models were developed and validated by DIRECT for diabetes to be used as screening tools by doctors.

In addition, this dissertation intends to present a joint vision of the IMI-projects with strategies for integrating personalized medicine into healthcare practice. This approach involves the creation of biological and genetic indicators that can be used to identify individuals at high risk of developing diabetes, the adoption of tools that allow early diagnosis and, lastly, the selection of appropriate treatment, i.e. the safest and most effective, supported by patient stratification models, in order to prevent/delay the development of diabetic complications.

Key-words: Innovative Medicines Initiative; Diabetes; Complications of diabetes; Personalized medicine.

Resumo

A Iniciativa sobre Medicamentos Inovadores é uma parceria público-privada entre a Comunidade Europeia, representada pela Comissão Europeia, e a Indústria Farmacêutica, representada pela Federação Europeia da Indústria Farmacêutica. Esta Iniciativa de Tecnologia Conjunta tem como objetivos acelerar o processo de investigação e desenvolvimento de medicamentos inovadores, bem como gerar novos conhecimentos científicos que promovam a integração da medicina personalizada nas doenças prioritárias definidas pela Organização Mundial de Saúde. Atualmente, no âmbito desta iniciativa foram estabelecidos dois programas, sendo que o primeiro (IMI1) decorreu entre 2008 e 2013 e teve um orçamento de 2 mil milhões de euros, enquanto que o segundo programa (IMI2) está em decurso desde 2014 e terminará em 2020 e o orçamento disponibilizado foi de 3.276 mil milhões de euros.

A diabetes mellitus é uma das doenças prioritárias indicadas pela Organização Mundial de Saúde alvo de financiamento pelos programas IMI. As principais justificações para este facto prendem-se com os dados epidemiológicos da doença. No decorrer dos anos, verificou-se um aumento exponencial da taxa de prevalência desta doença a nível mundial. Esta evidência é suportada pelo facto de, entre o período de 1980 e 2014, esta taxa ter sofrido um aumento de 4.7% para 8.7%, o quadruplo do valor, em adultos com idade igual ou superior a 18 anos e também por as estimativas a 20 anos, realizadas pela Organização Mundial de Saúde, indicarem que o número total de casos existentes corresponderá a mais de 20% da população universal. Simultaneamente, constatou-se um crescimento progressivo tanto da taxa de mortalidade por diabetes bem como dos custos de saúde acarretados por esta doença. No que diz respeito à taxa de mortalidade, em 2016, a diabetes foi considerada a sétima principal causa de morte no mundo. Em termos de impacto económico, a diabetes e as complicações decorrentes desta doença, como é o caso das doenças cardiovasculares, nefropatia diabética e retinopatia diabética, impõem um grande peso económico para os sistemas de saúde. A nível mundial, os custos anuais provocados pela diabetes, entre o ano de 2007 e 2019, aumentaram de 232 mil milhões de dólares para 760 mil milhões de dólares, o que equivale a incremento de 528 mil milhões de dólares em 12 anos.

Na área da Diabetes, o principal objetivo dos programas IMI1 e IMI2 é o de reduzir a tendência crescente observada na taxa de prevalência desta doença. De forma a

atingir esta meta, os dois programas supramencionados primaram o financiamento de projetos cujo intuito consistia no desenvolvimento do conhecimento, medicamentos, métodos, ferramentas e modelos que facilitassem a implementação da medicina personalizada, como modelo de prática médica corrente, em doentes com diabetes. Até outubro de 2019, os programas IMI financiaram treze projetos para a área da Diabetes & Doenças Metabólicas, nomeadamente SUMMIT, IMIDIA, DIRECT, StemBANCC, EMIF, EBiSC, INNODIA, RHAPSODY, BEAT-DKD, LITMUS, Hypo-RESOLVE, IM2PACT e CARDIATEAM. Entre estes, o INNODIA tinha como objetivo a diabetes tipo 1, o DIRECT, EMIF e RHAPSODY tinham como foco a diabetes tipo 2, o SUMMIT, BEAT-DKD, LITMUS, Hypo-RESOLVE e CARDIATEAM estavam associados às complicações da diabetes e os restantes projetos, o StemBANCC, EBiSC, IMIDIA e IMI2PACT, estavam orientados para o desenvolvimento da vertente científica.

Em geral, um investimento monetário total na ordem dos €447 249 438 foi realizado pelo IMI na área da Diabetes. Todavia, a deteção da lacuna existente na integração dos resultados produzidos pelos diferentes projetos, impulsionou a elaboração da presente dissertação intitulada de "Scientific Advances in Diabetes: The Impact of the Innovative Medicines Initiative", ou seja, Avanços Científicos na Área da Diabetes: Impacto da Iniciativa sobre Medicamentos Inovadores.

Os principais objetivos estabelecidos para esta dissertação foram os de recolher os artigos publicados pelos projetos financiados e sistematizá-los nos eixos de investigação definidos na agenda estratégica do programa IMI2, mais concretamente: 1) identificação de alvos e biomarcadores, 2) novos paradigmas de ensaios clínicos, 3) medicamentos inovadores e 4) programas de adesão terapêutica centrados nos doentes.

A metodologia de investigação aplicada nesta dissertação consistiu numa revisão de literatura, tendo-se utilizado como fontes de dados as páginas eletrónicas oficiais de cada projeto, o contacto com os coordenadores e co-coordenadores dos projetos e a base de dados europeia Cordis. No geral, um total de 662 citações foram identificadas, das quais 185 foram incluídas na análise realizada neste trabalho.

Através da sistematização e integração dos artigos recolhidos nos projetos financiados pelo IMI, averiguou-se que para o eixo de identificação de alvos e biomarcadores, os *outcomes* relevantes responderam a cinco das recomendações

definidas na agenda estratégica do programa IMI2, nomeadamente: 1) identificar e validar marcadores biológicos, ferramentas e ensaios, 2) identificar as determinantes que justificam a variabilidade interindividual, 3) compreender os mecanismos moleculares subjacentes à doença, 4) desenvolver uma plataforma de ensaios pré-clínicos e 5) estabelecer modelos de sistemas. De um modo geral, um vasto número de biomarcadores, ferramentas, fatores responsáveis pela heterogeneidade da população, incluindo marcadores genéticos, e mecanismos relevantes foram identificados para a diabetes tipo 1 pelo INNODIA, para a diabetes tipo 2 pelo SUMMIT, IMIDIA, DIRECT e EMIF, para as células beta pancreáticas pelo IMIDIA e RHAPSODY, para a nefropatia diabética pelo SUMMIT e BEAT-DKD, e para as doenças cardiovasculares e retinopatia diabética pelo SUMMIT. Suplementarmente, um conjunto de ferramentas e ensaios foram desenvolvidos pelos projetos StemBANCC, EBiSC e IMIDIA com o intuito de impulsionar avanços na área investigacional. Ainda neste eixo foram propostos dois modelos de estratificação dos doentes, um relativo ao controlo glicémico em doentes com diabetes tipo 1 estabelecido pelo INNODIA e outro correspondente à identificação dos subtipos de doentes com diabetes desenvolvido pela parceria BEAT-DKD/RHAPSODY.

Relativamente ao eixo de ensaios clínicos, os dados analisados compreendiam propostas de novos parâmetros clínicos e de desenhos de ensaios, sendo que estes resultados visavam espelharem com maior precisão as características da subpopulação com diabetes em teste. Os dados incluídos neste eixo foram obtidos a partir dos projetos SUMMIT, DIRECT e BEAT-DKD.

No que concerne ao eixo de medicamentos inovadores, as informações recolhidas dos artigos publicados pelos projetos SUMMIT, IMIDIA, DIRECT, StemBANNC, EMIF, INNODIA e BEAT-DKD consistiam na identificação de novos potenciais alvos terapêuticos bem como no desenvolvimento de novos agentes terapêuticos, ambos com a finalidade de tratar ou prevenir tanto a diabetes como as complicações associadas a esta doença. Foi ainda proposta uma nova abordagem de produção de células estaminais pluripotentes humanas em larga escala pelo StemBANCC.

No que tange aos programas de maximização de resultados de saúde benéficos centrados no doente com diabetes, dois novos modelos preditivos foram desenvolvidos e validados pelo projeto DIRECT, permitindo a sua utilização como ferramentas de diagnóstico por médicos especialistas.

Adicionalmente, esta dissertação tem como objetivo apresentar uma proposta de visão de complementaridade entre os treze projetos financiados pelo IMI, realçando as possíveis estratégias a adotar para a integração da medicina personalizada na prática clínica. Esta abordagem engloba a criação de indicadores biológicos e genéticos que facilitem a identificação dos indivíduos com risco elevado de desenvolver diabetes, a inclusão de ferramentas que possibilitem o diagnóstico precoce dos doentes e, por último, a seleção do tratamento apropriado às características do indivíduo, ou seja o que evidencie ser mais eficaz e seguro, suportado em modelos de estratificação de doentes, tentando desta forma retardar a progressão da doença, assim como prevenir o desenvolvimento das complicações relacionadas com a progressão da doença.

Palavras – chave: Iniciativa sobre Medicamentos Inovadores; Diabetes; Complicações da Diabetes; Medicina Personalizada.

Acknowledgements

To Professor Maria Beatriz da Silva Lima,

Professor Carla de Matos Torre,

my whole family,

and my friends,

thank you.

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List of Abbreviations

ABACUS – Algorithm based on a Bivariate Cumulative Statistic

ADHD – Attention-Deficit Hyperactivity Disorder

BBB – Blood-Brain Barrier

BCAA – Branched-Chain Amino Acids

BEAT-DKD – Biomarker Enterprise to Attack DKD Project

BiPSC – β-cell derived iPSCs

Bi-DOCS – BiPSC Specific Differential Open Chromatin Sites

BMI – Body Mass Index

BoNB – Bag of Naïve Bays

BOSS – Binary Outcome Stochastic Search

CARDIATEAM – Cardiomyopathy in Type 2 Diabetes Mellitus Project

CGM – Continuous Glucose Monitoring

CORDIS – Community Research and Development Information Service

CTD – Common Technical Document

CVD – Cardiovascular diseases

CYP2C9 – Cytochrome P450 2C9

DBS – Dried Blood Spots

DIRECT – Diabetes Research on Patient Stratification Project

DKD – Diabetic Kidney Disease

DM – Diabetes Mellitus

DN - Diabetic Nephropathy

DR - Diabetic Retinopathy

EBiSC – European Bank for induced pluripotent Stem Cells Project

EC – European Community

EFPIA – European Federation of Pharmaceutical Industries and Associations

eGFR – Estimated Glomerular Filtration Rate

EMIF – European Medical Information Framework Project

EU – European Union

ER – Endoplasmic Reticulum

E&T – Education and Training

FDA – Food and Drug Administration

FPG – Fasting Plasma Glucose

GADA – Glutamic Acid Decarboxylase Autoantibodies

GBR – Genotype-based Recall

GDP – Gross Domestic Product

GGT -Gamma-Glutamyl Transpeptidase

GLP1R – GLP1 Receptor

HbA_{1c} – Glycated Haemoglobin

HDL – High-Density Lipoprotein

HNB – Hierarchical Naïve Bayes

HPSCs – Human Pluripotent Stem Cells

HTA – Health Technology Assessment

Hypo-RESOLVE – Hypoglycaemia - Redefining Solutions for Better Lives Project

ICH – International Council for Harmonisation

IDF – International Diabetes Federation

IGF-1 – Insulin-like Growth Factor 1

IGT – Impaired Glucose Tolerance

IMI – Innovative Medicines Initiative

IMI1 – First Innovative Medicines Initiative programme

IMI2 – Second Innovative Medicines Initiative programme

IM2PACT – Investigating Mechanisms and Models Predictive of Accessibility of Therapeutics into the Brain Project

IMIDIA – Improving Beta-cell Function and Identification of Diagnostic Biomarkers for Treatment Monitoring in Diabetes Project

INNODIA – Translational Approaches to Disease Modifying Therapy of Type 1 Diabetes: An Innovative Approach Towards Understanding and Arresting Type 1 Diabetes Project

iPS – Induced Pluripotent Stem

iPSCs – Induced Pluripotent Stem Cells

KM – Knowledge Management

LEAD – Lower Extremity Arterial Disease

LITMUS – Liver Investigation: Testing Marker Utility in Steatohepatitis Project

MA – Marketing Authorisation

MARD – Mild Age-related Diabetes

MEHFMRI – Manganese-Enhanced High Field Magnetic Resonance Imaging

MG – Methyl-Glyoxyl

MOD – Mild Obesity-related Diabetes

MODY – Maturity-Onset Diabetes of the Young

MUFAs – Monounsaturated Fatty Acids

NAFLD – Non-Alcoholic Fatty Liver disease

NASH – Non-alcoholic Steatohepatitis

NDA – New Drug Application

NFAT – Nuclear Factor of Activated T-cells

NMEs – New Molecular Entities

OCT – Optical Coherence Tomography

OCT1 – Organic Cation Transporter 1

OGTT – Oral Glucose Tolerance Test

PPP – Public and Private Partnership

RAAS – Renin-Angiotensin-Aldosterone System

RC – Redifferentiation Cocktail

RHAPSODY – Assessing Risk and Progression of Prediabetes and Type 2 Diabetes to Enable Disease Modification Project

R&D – Research and Development

SAID – Severe Autoimmune Diabetes

SHBG – Sex Hormone-Binding Globulin

SIDD – Severe Insulin-Deficient Diabetes

SIRD – Severe Insulin-Resistant Diabetes

SMBG - Self-Monitoring of Blood Glucose

SME – Small and Medium-Sized Enterprise

SNP – Single nucleotide polymorphism

SRA – Strategic Research Agenda

StemBANCC – Stem Cells for Biological Assays of Novel Medicines and Predictive Toxicology Project

SUMMIT – Surrogate Markers for Micro- and Macro-Vascular Hard Endpoints for Innovative Diabetes Tools Project

T1D – Type 1 diabetes

T2D – Type 2 diabetes

T2D-PLN – Type 2 Diabetes phenotypic-linkage network

UACR – Urinary Albumin Creatinine Ratio

UPSA – Ultrasound Plaque Structure Analysis

US – United States

VEGFA – Vascular Endothelial Growth Factor

VLDL – Very-Low-Density Lipoprotein

WAT - White Adipose Tissue

WHO – World Health Organization

Introduction

Innovative Medicines Initiative

Innovative Medicines Initiative is a unique pan-European public and private partnership (PPP) that pioneered large-scale open collaborations between large pharmaceutical companies, small and medium-sized enterprises (SME), public authorities (including regulators), organizations of patients, academia and clinical centres, to throw bottlenecks in research and development (R&D) of new effective and safer medicines (1), as presented in Figure 1. This should provide socio-economic benefits and contribute to the health of European citizens, increase the competitiveness and help establish Europe as the most attractive and competitive site in R&D (2). Figure 2 consists of IMI's logo.

Figure 1 - IMI intends to operate in areas where the combination of research, regulatory, healthcare and other relevant stakeholders is crucial to the R&D process of innovative medicines and drive towards precision medicine. Source: Strategic Research Agenda for Innovative Medicines Initiative 2.

Figure 2 - Logo of the Joint Technology Initiative on Innovative Medicines.

The Joint Technology Initiative on Innovative Medicines arose in response to the following calls for action (2):

- Commission Communication of 1st of July 2003 ‘A Stronger European-based Pharmaceutical Industry for the Benefit of the Patient — A Call for Action’;
- Report ‘Stimulating Innovation and Improving the EU Science Base’ adopted on 7th of May 2002;
- Commission Communication of 23rd of January 2002 ‘Life Sciences and Biotechnology — a strategy for Europe (2002)’;
- Report ‘Creating an Innovative Europe’ of January 2006.

The first programme of IMI (IMI1) was created by Council Regulation (EC) no. 73/2008, of 20th December 2007. The overall aim of this joint initiative was to support pre-competitive pharmaceutical research and development, through the funding of innovative patient-centred projects for the research of priority diseases, in the Member States and countries associated with the Seventh Framework Programme (2).

From a generalist point of view, the traditional medicine R&D process englobes the identification and validation of potential therapeutic targets for disease, discovery of the appropriate molecule (potential medicine) to interact with the target and test it in non-clinal models (*in-vitro* and animals) to collect data regarding its pharmacology, pharmacokinetics and toxicology (3). Therefore, in the case of the potential medicine proving to be effective and safe, it should initiate clinical trials I, II and III, and in the presence of supportive data of quality, efficacy, safety and a positive benefit-risk, the pharmaceutical company submits to the competent authority a Common Technical Document (CTD) or New Drug Application (NDA), according to International Council for Harmonisation (ICH) and Food and Drug Administration (FDA) terminology, respectively (1,4). After gaining approval, it follows the scale-up to industrial manufacture and post-marketing surveillance (pharmacovigilance). This is a long process, taking an average of 10 to 15 years, as well as complex and resource-intensive, as illustrated in Figure 3.

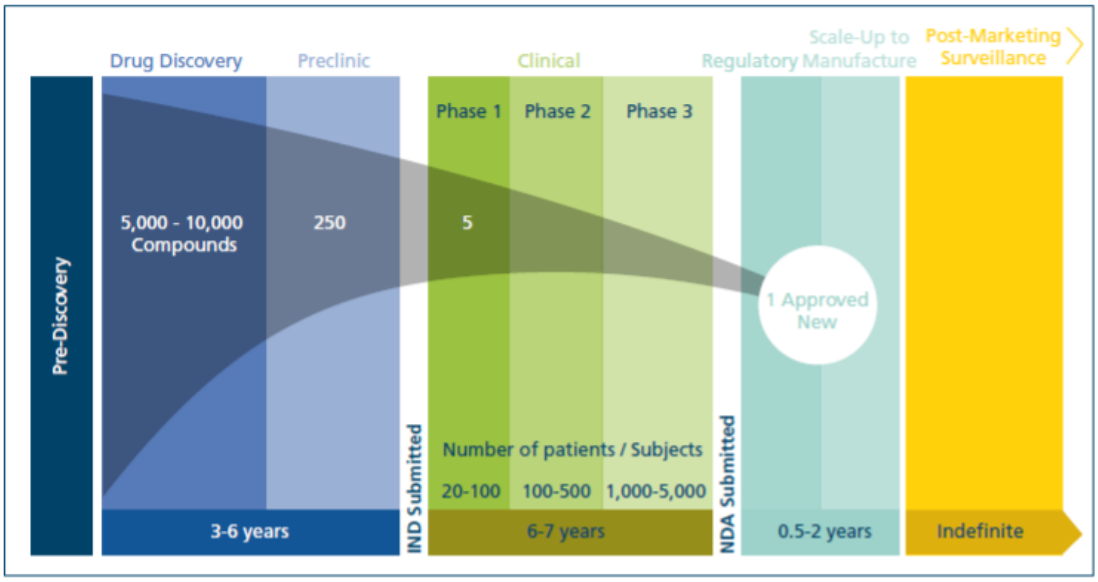

Figure 3 - Overall process of medicine research and development and timeline. Source: The Innovative Medicines Initiative Research Agenda.

During the period of 1994 to 2007, the cost of medicines' R&D presented a gradual growth, in Europe and the United States (US), as presented in Figure 3. The estimated costs in 2007 were of ≈26.000 million euros and ≈35.000 million US dollars, respectively (5).

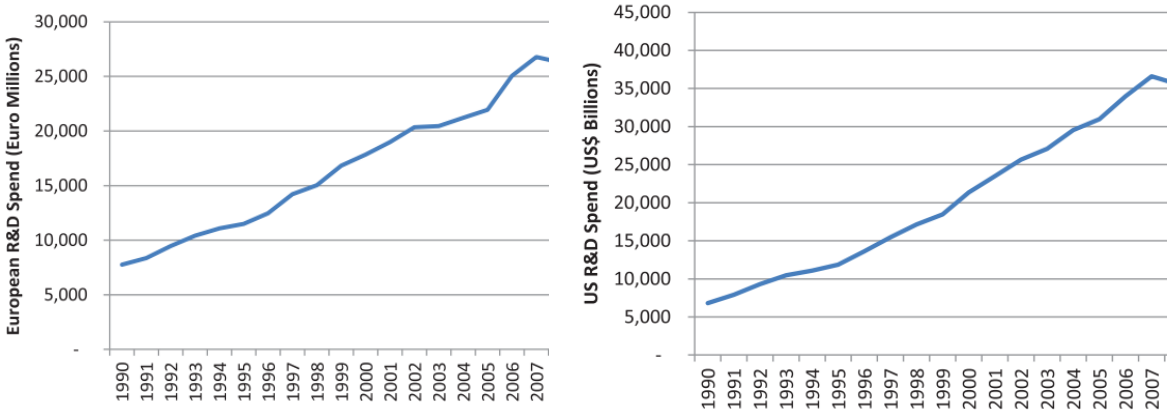

Figure 4 - European and US medicine R&D Spending between 1990 and 2007. Source: EFPIA.

Although the general trend of R&D costs was a rising one, a similar increase was not verified in the number of new molecular entities (NMEs) launched. According to European Federation of Pharmaceutical Industries and Associations (EFPIA) data, exhibited in Table 1, after the period of 1990-1994, the R&D productivity declined since the number of NMEs diminished from an average of 43 per year to ≈30 or fewer per year until 2007 (5).

Table 1 - Number of new molecular entities from 1990 to 2007.

Timeline	*1990–1994*	*1995–1999*	*2000–2004*	*2005-2007*
Average of NMEs per year	43	41	32	30

Besides, the attrition rates were very high, with a success rate from pre-clinical stages to market ≤6% (1,5). The major factors identified for project failure were lack of efficacy (25%), clinical safety concerns (12%) and toxicological findings in pre-clinical evaluation (20%) (1). As a consequence, there was a massive necessity of reducing the risk of unsuccessful projects and simultaneously predict failure at the earliest possible stage of the medicine development process, through the improvement of the predictivity of pre-clinical studies to anticipate clinical safety and clinical efficacy, as well as the overall assessment of patient benefits and risks with regulatory authorities (1).

Following this line of thought, the IMI1 programme was based on four strategic interdependent areas (Four-pillars), namely Safety, Efficacy, Knowledge Management (KM), and Education and Training (E&T), regarded as important areas, as presented in Figure 5 (1).

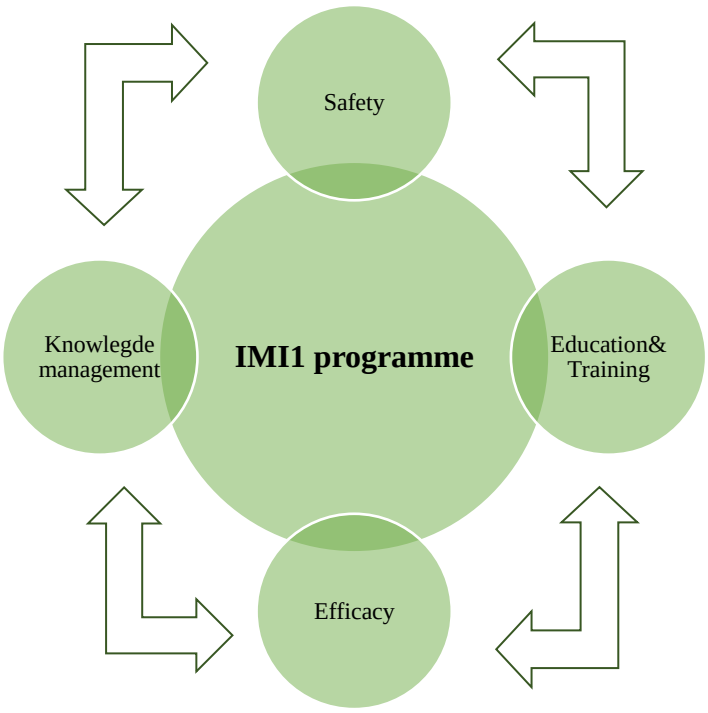

Figure 5 - Four-Pillars established for the IMI1 programme.

The vision of this programme consisted on the creation of new scientific knowledge and capabilities/techniques to support the ability to identify a lack of efficacy or safety quickly in all stages of the medicine development process, even when a potential medicine has promising pre-clinical data. Besides, IMI1 programme intends to support the benefit-risk assessment conducted by the regulatory authorities, as illustrated in Figure 6.

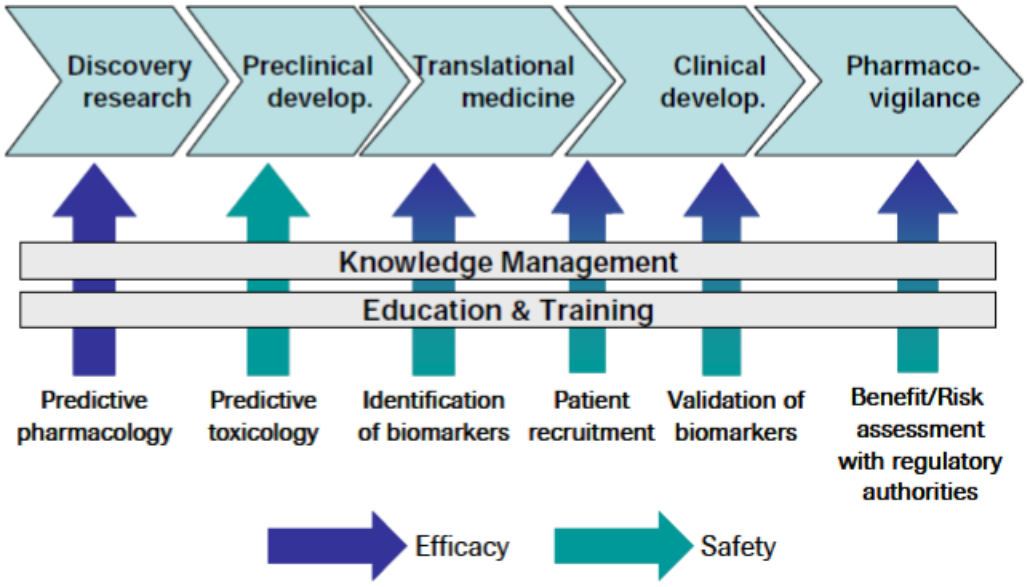

Figure 6 - Vision of IMI1 programme and interdependency between the Four-Pillars established for this programme. Source: The Innovative Medicines Initiative Research Agenda.

Additionally, IMI1 programme created the opportunity to drive the concept of personalised medicine (Figure 7), which consists in anticipating strategies for individualized treatment decisions based on biological, personal, environmental and social factors, enabling patient's stratification and consequently selection of the best treatment, i.e. the safest and most effective, and most-appropriate dosage (1).

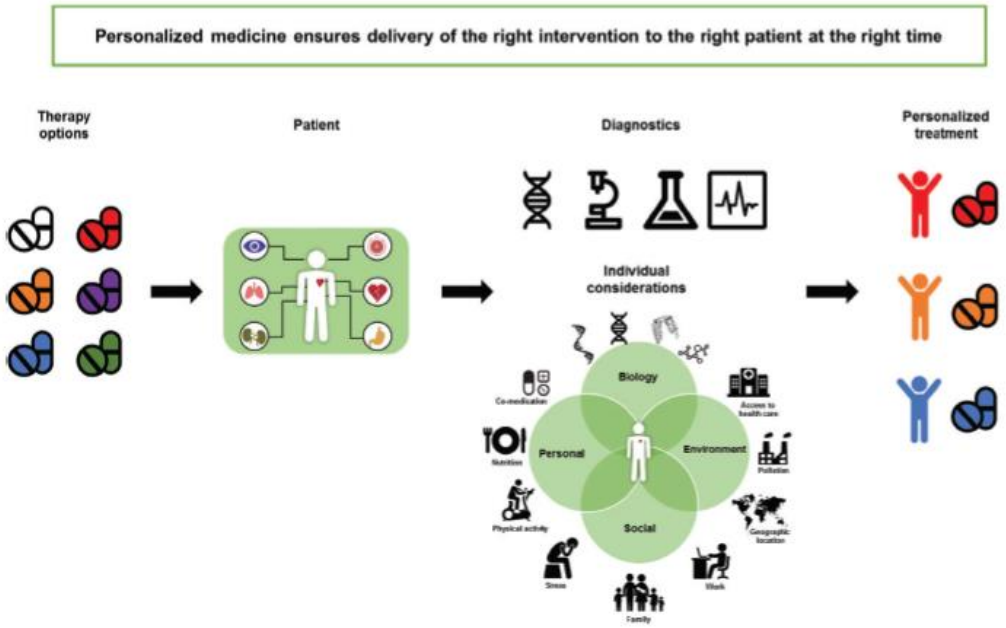

Figure 7 - Implementation of personalized medicine. Source: Vries, J., Levin, A., Loud, F., Adler, A., Mayer, G. Pena, M. (2018). Implementing personalized medicine in diabetic kidney disease: Stakeholders' perspectives, Diabetes, Obesity and Metabolism, Wiley, 20(Suppl. 3):24–29.

Pillar I "Safety" addressed bottlenecks related to predictivity in safety evaluation and benefit-risk assessment with regulatory authorities. With the purpose of improving this factor, the Strategic Research Agenda (SRA) presented nine recommendations/goals, as described in (1):

- Create a European Centre of Drug Safety Research to identify and co-ordinate research needs in safety sciences;
- Establish a framework to develop biomarkers that will indicate the human relevance and regulatory utility of early laboratory findings;
- Study the relevance of rodent non-genotoxic carcinogens;
- Develop *in silico* methods for predicting conventional and recently recognized types of toxicity;
- Explore the implications of intractable toxicity in animals for human risk;
- Optimize data resources and strengthen the evidence base in pharmacovigilance;
- Develop and strengthen methodologies and networks for pharmacovigilance;
- Develop novel methods of risk prediction and benefit-risk assessment;
- Train and educate health care professionals and patients.

Pillar II “Efficacy” addressed bottlenecks in medicine development related to predictive pharmacology, the identification, and validation of biomarkers, criteria of patient recruitment as well as benefit-risk assessment with regulatory authorities. For this set, SRA identified the following nine recommendations/goals, as described in (1):

- Develop a better understanding of disease mechanisms;
- Develop *in vitro* and *in vivo* models predictive of clinical efficacy;
- Develop *in silico* simulations of disease pathology;
- Stimulate translational medicine in an integrated fashion across industry and academia;
- Create disease-specific European Imaging Networks to establish standards, ensure imaging biomarkers are validated, and develop regional centres of excellence;
- Create disease-specific European Centres for the validation of omics-based biomarkers;
- Co-ordinate the development of national patient networks and databases to develop a true pan- European organization for patient selection and clinical trial analysis;
- Form a European stakeholder consortium to address value demonstration, including quality of life issues, patient-reported outcomes and the burden of disease;
- Develop a partnership with regulators to devise innovative clinical trial designs and analyses, to aid acceptance of biomarkers and to promote data sharing and the joint consideration of ethical issues.

Pillar III “Knowledge Management” addressed bottlenecks related to gaps in information in the R&D process. Concerning this axis, fifteen recommendation/goals were recognised by SRA, as described in (1), namely:

- Set up a Translational KM team to support individual Safety and Efficacy projects, to define standards of compatibility across projects, and to promote the sharing of suitable KM technology;
- Set up a KM Platform team that, through partner consortium projects, conceives the overall architecture for an integrating biopharma/biomedicinal sciences platform;

- Set up an advisory Science Panel that supports the KM team in applied Information Technology matters, the ongoing evaluation of prior art, and the identification of complementary and synergistic technology R&D proposals;
- Set up task forces to evaluate cross-disciplinary aspects such as modelling and simulation of physio-pathological processes, validate specifications, and align priorities;
- Set up a cross-disciplinary task force to propose guidelines concerning non-KM issues related to data sharing, for example legal, regulatory, ethical and intellectual property;
- Evaluate the approaches and the investment required to build the core of a platform backbone ontology;
- Develop enhanced standards for data protection in a web services environment;
- Develop standards and models for exposing web services (semantics), scientific services, and the properties of data sources, datasets, scientific objects, and data elements;
- Develop enhanced knowledge representation models and data exchange standards for complex systems which, at present, are largely lacking, inconsistent, or incomplete, looking for synergies with current initiatives;
- Develop new, domain-specific ontologies, built on established theoretical foundations and considering current initiatives, existing standard data representation models, and reference ontologies;
- Develop advanced text mining tools for capturing implicit information about complex processes, as described in patents and the literature, beyond and above simple pair-wise relationships between entities;
- Develop innovative and powerful data exploitation tools, for example, multi-scale modelling and simulation, considering and integrating from the molecular to the systems biology level, and from the organ to the living organism level;
- Build a core reference database of validated experimental data extracted from the literature;
- Design standards for and build an expert tool (ontology/schema/rules negotiator) for exposing the properties of local sources in a federated environment;

- Design standards for an expert tool (services/data negotiator) to guide users through the complexities of the data, data models, simulation and modelling tools and so on.

Pillar IV "Education and Training" addressed the bottlenecks related to gaps in expertise in biomedical R&D knowledge and capabilities that will impact on medical practice. The five recommendations defined by SRA, as described in (1):

- Establish a European Medicines Research Academy, including a central co-ordinating unit and an advisory Education and Training council;
- Establish programmes for integrated medicines development and ethics committees and patient organizations;
- Establish programmes for safety sciences, scientists within pharmaceutical R&D and pharmaceutical medicine professionals;
- Establish regulatory affairs-based programmes;
- Establish programmes for biostatisticians, bioinformaticians and biomedical informaticians.

In summary, thirty-eight multi-disciplinary recommendations/goals were defined within the Four-Pillars of the SRA, to be taken into account by the projects. Besides the several areas addressed by IMI previously mentioned, it is extremely important to also highlight the impact of IMI on the use of animals in the R&D process. This programme intends to support the concept of the '3Rs': Replacement, through the substitution of animals with valid non-animal models such as *in vitro* and *in silico* techniques; Reduction, by the use of methods, as biomarkers, that allow the necessary information to be obtained from fewer animals; and Refinement, by using methods which cause the least possible animal distress (1).

For IMI1 programme the budget committed was €2 billion (6). During the execution period of IMI1 programme (2008 to 2013), eleven calls for proposals were released, which resulted in fifty-nine funded-projects (6).

The results achieved by the projects funded under the IMI framework were focussed on the development of new biological markers and health outcome measures, improving the ability to gather patient data, quantify and communicate the benefit/risk of

medicines, improving the ability to predict the safety of new medicines, and enhancing the ability to translate pre-clinical pharmacology to the clinical setting (7).

The success of IMI1 programme prompted the European Commission and EFPIA to take the commencing of initiating a second IMI programme (IMI2) under the Horizon 2020 vision of "improve the health and well-being of populations, reduce health inequalities, and ensure sustainable people-centred health systems" (7). See Figure 8.

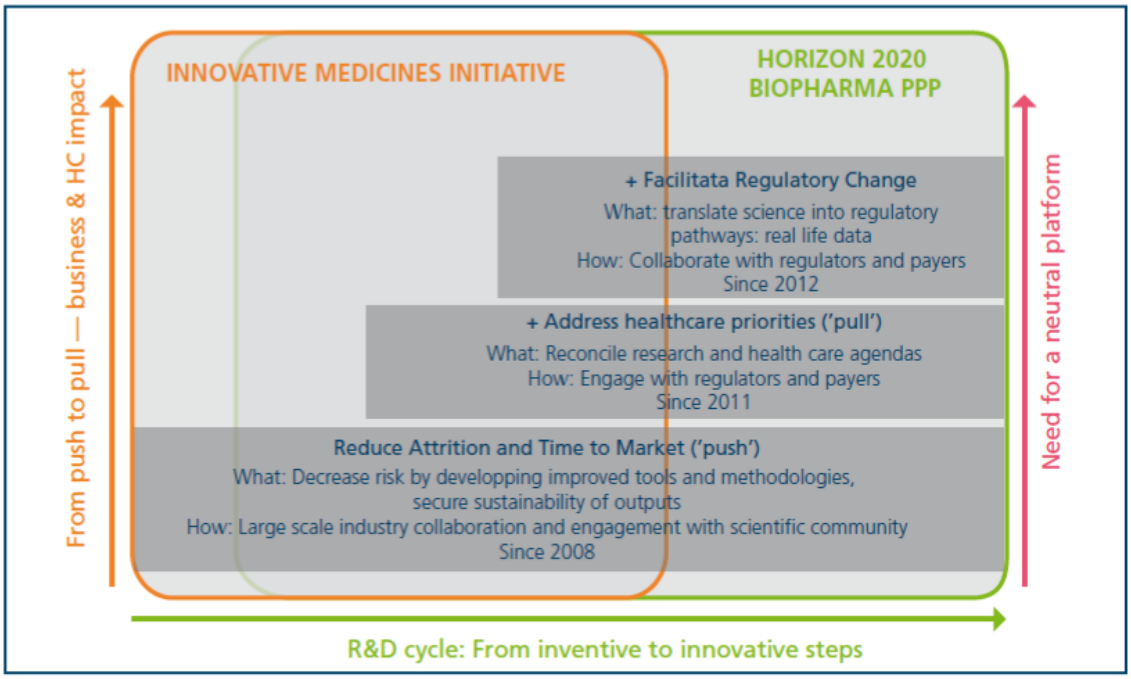

Figure 8 - Transition from IMI1 programme to IMI2 programme according to the goals of Horizon 2020. Source: Strategic Research Agenda for Innovative Medicines Initiative 2.

Innovative Medicines Initiative 2 Joint Undertaking was established by Regulation (EU) on 557/2014, 6th of May, with the objectives of increasing the success rate in clinical trials of priority medicines identified by the World Health Organization (WHO), reducing the time to reach clinical proof of concept in medicine development, developing new therapies for diseases for which there are a high unmet need and limited market incentives, developing diagnostic and treatment biomarkers for diseases linked to clinical relevance and approved by regulators, developing biomarkers for initial efficacy and safety checks of vaccine candidates in phase III clinical trials, and improving the current medicine development process by providing support for the development of tools, standards and approaches to assess efficacy, safety and quality of regulated health products (8).

The vision of IMI2 programme was to deliver tools, methods and treatment options that would promote personalised medicine and prevention (7). The execution period established was between 2014 to 2020 and the budget committed was up to €3.276 billion (6).

In IMI2 programme, the major research axes recognized as the pillars to address the increase of the success probability and reducing the overall cost of new medicines were: 1) target & biomarker identification, 2) innovative clinical trial paradigms, 3) innovative Medicines and 4) Patient tailored adherence programmes (7), as presented in Figure 9.

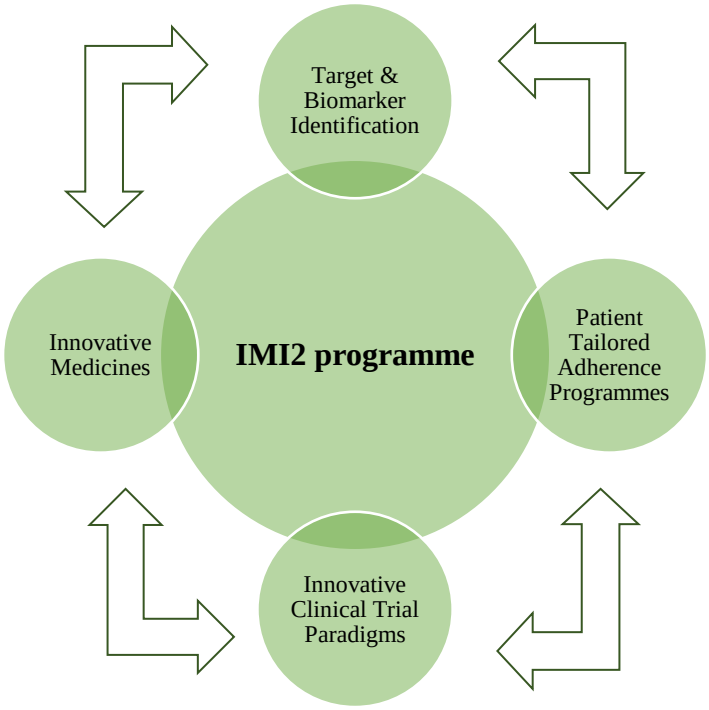

Figure 9 – Four major axes established for theIMI2 programme.

Axis 1 "Target & Biomarker Identification" addressed the failure of efficacy in translating pre-clinical models to the clinical setting, the occurrence of adverse events not expected from the pre-clinical models, and the opportunity to drive the concept of precision medicine. The following ten objectives were identified by SRA for this axis, as described in (7):

- Identify new or alternative therapeutic concepts (targets) for treatment and prevention of diseases such as the study of endophenotypes and the effects of pharmacogenetics on disease progression and treatment. Further, it will generate the research tools (e.g. chemical probes, recombinant antibodies, systems analysis) required to further characterise the biology of novel genes/proteins/cells and validate new therapeutic concepts pre-clinical and clinically;
- Identify and validate biological markers, tools and assays (biochemical, functional and imaging) to support disease reclassification and patient stratification approaches, monitor disease progression, provide proof of pharmacological response, predict and monitor the efficacy and safety of medicines and vaccines, as well as biomarkers that may serve as surrogate markers in clinical trials;
- Better understand the types of biomarkers, outcomes and composite endpoints that regulators and Health Technology Assessment (HTAs) could accept and what level of validation is needed for their utilisation in order to direct discovery efforts. Initiate formal consultation procedures as appropriate;
- Enhance understanding of the immunological mechanisms and host-pathogen and host–vaccine interactions to enable improvements in the design of both preventive and therapeutic vaccines;
- Improve the profiling of immune responses to infection and to vaccination in different age groups, identifying novel correlates of protection against infectious diseases and possibly other non-infectious conditions;
- Better understanding of the molecular determinants of inter-individual variability to medicine and vaccine efficacy and safety thus reducing the underlying biological variability of trial patient populations to enable reliable measures of treatment effect;
- Understand the molecular mechanisms underlying medicine toxicity in humans to drive mechanism-based medicine and vaccine safety assessment and early prediction of clinical and non-clinical medicine and vaccine response to improve the predictability of translating pre-clinical findings to the clinical setting;
- Develop non-invasive measures (such as imaging technology) of medicine exposures at the organ level to deliver a better understanding of the pharmacokinetics/ pharmacodynamics relationship of a medicine or vaccine and therefore more accurately predicting the therapeutic index of a medicine;

- Develop a platform of pre-clinical assays utilising normal and disease tissue, cell lines, stem cell technology, genetic manipulation and cloning to create more predictive *in vitro*, *ex vivo* and *in vivo* models of the relationship between medicine exposure, pharmacological response, inter-individual variability with respect to efficacy and safety to improve translation from pre-clinical testing to the clinic;
- Develop systems models and strategies combining technology, biology (omics) and computational methods, with information retrieved from historical compounds tested in preclinical models or patients for evaluation/prediction of medicine safety and efficacy.

Axis 2 "Innovative clinical trial paradigms" attempted to decrease clinical development times and costs as well as minimise the uncertainty for regulatory authorities on the safety and efficacy of new medicines. The twelve core objectives established by SRA, as described in (7), for this set were:

- Establish a framework (with clearly defined rules, ethical standards and data privacy measures) to support the interaction of key stakeholders (especially patient groups) involved in the clinical development of new medicines and vaccines. Principles will be applied across all axes of research.
- Develop innovative clinical endpoints which more closely reflect value to the patient and HTAs (e.g. patient-reported outcome measures) and better understand the types of composite endpoints that regulators and payers could accept and what level of validation is needed for their utilisation initiating formal consultation as required;
- Evaluate the economic benefit of new diagnostic and health outcome measures across different healthcare systems;
- Employ advances in digital media to develop new methods of collecting benefit/risk data and standardise methods across different countries;
- The development of predictive pre-clinical tools for toxicity and/ or immunogenicity to reduce the risk of failure in clinical trials. Increase the role of the patient in research by including patient-reported outcomes, improve the methodologies available for communicating the benefit/risk of new medicines and vaccines, thus creating a community approach to pharmacovigilance. Understanding patient preferences, real-life use and effectiveness, the efficiency

of risk mitigation strategies and caregiver experience through direct questioning in trials, and through observed comment in social media, diaries and other less direct ways;

- Validate quantitative methodologies for assessing patient and healthcare professionals' preferences in benefit/risk assessments pre- and post-market authorisation. Establish agreement on how best to integrate patient preferences with regulatory benefit/risk criteria and effectiveness/risk assessment and clinical judgement across different countries;
- Utilise innovative endpoints, trial designs, simulation and analytical approaches to devise new clinical trial paradigms both pre- and post-marketing which permit the assessment of outcomes (good and bad) in small patient populations balancing the needs for regulation (efficacy/safety) and HTA agencies (effectiveness/ safety) as well as the risk and cost for pharmaceutical companies;
- Establish validated systems to support the cost-effective collection of high-quality benefit/risk and effectiveness/risk data via digital media and understand how this can be integrated into pharmacovigilance framework;
- Continue to build on efforts already underway in EHR4CR and Transcelerate, to harness the use of electronic healthcare record systems to improve the design and conduct of randomised clinical trials, pragmatic and adaptive and extend these efforts to support the conduct of efficient and high-quality observational research on medicines and full care pathways after medicines are authorised and used in clinical practice;
- Gain agreement on the pre-approval data required for conditional marketing authorisation (MA) vs. 'full' MA, qualitative vs. quantitative assessment methodology, and the need for active comparator clinical trials; post-approval data required to maintain the benefit/risk assessment; alignment on data required for regulatory benefit/risk and HTA needs; and communication of benefit/risk within medicine labels;
- Create the framework required to successfully implement "Medicines Adaptive Pathways for Patients" to provide a basis for the effective and safe introduction of new treatments for selected populations. Work proactively with payers early in the life cycle to agree on how a product should be reimbursed when utilising the MAPP framework;

- Build on efforts currently ongoing in GetReal to create a framework to allow the identification of and overcome the operational difficulties associated with generating evidence of relative effectiveness before launch and provide proof of concept for new regulatory pathways to inform discussion on regulatory guidance.

Axis 3 “Innovative Medicines” addressed the need that arose from the ageing population and increased incidence of chronic diseases, mainly in terms of investment in developing new therapeutic strategies that integrate early detection and prevention. For this area, the following eleven objectives were established by SRA, as described in (7):

- Where the burden of disease is high, conduct the research required to determine interventions that would provide the biggest improvement to patient health outcomes from both a patient and healthcare provider perspective, establish the potential economic benefit of intervention and impact on healthcare delivery;
- Better understand the barriers to investment of pharmaceutical companies where the burden of disease is high and return is low, and develop creative approaches to incentivise research in these disease areas;
- Conduct the basic research and develop the tools required to support the development of innovative preventative medicines for the disease of high societal impact;
- Implement new approaches for the development and production of biopharmaceuticals, vaccines, cell-based therapies, tissue engineering, gene therapies, preventive medicines or more rapid diagnostics;
- Where co-investment is justified based on societal and healthcare need, and there are sufficient validated tools available, jointly develop novel therapeutic agents and disease prevention strategies;
- Conduct research required to support the establishment of the necessary regulatory pathways and frameworks and payer framework to support the authorisation of new preventative medicines (driven under axis 2);
- Conduct research in manufacturing technology to produce innovative medicines through highly flexible and cost-effective processes that guarantee high quality and safety;
- Develop new simple and robust process and product analytical tools to assure highly controlled and safe production processes, including effective methods to detect and prevent adventitious agent contaminations;

- Develop and/or optimize vaccine/protein formulation and conduct more research for the right excipients that increase stability, especially with regard to proteins and the new and complex multivalent vaccines.
- Provide improved access to information and support allowing individuals to make more informed decisions on the management of their own health and treatment options better understand individual behaviours which are shown to lead to health problems, and have potential opportunities for earlier, non-medical interventions;
- Integrate the needs of HTA agencies into the early R&D process, thus ensuring that at the time of the initial assessments of new medicine, the public sector review bodies have the data that support their robust evidence-based decision-making and R&D focus efforts on medicines that demonstrate sufficient value to warrant a reimbursement.

Axis 4 “Patient tailored adherence programmes” intended to maximise beneficial health patient-centred outcomes and reduce the incidence of non-adherence with prescribed medicines through the understanding of key areas such as pharmacology and perception, individual patient behaviours, social, lifestyle and environmental factors that influence the engagement of patients with their treatment pathways. Seven specific objectives were defined by SRA, as described in (7), more precisely:

- Conduct the research required to understand citizen and patient behaviours (for example failure to make lifestyle changes or adhere to prescribed medicine dosing regimens), develop and implement tailored solutions (along the continuum of education and prevention) that will enable patients and citizens to play a more active role in the management of their own health and treatment options;
- Integrate data sources pertaining to real-life use of medicines to support the creation of models to predict patient adherence based on risk factors and demographics to ensure new therapeutic opportunities have programmes to provide tailored follow-up and support.
- Collect information on medicines tailored to the needs of patients;
- Address the technology demands associated with precision medicines and vaccines:
    a. Develop innovative delivery systems, which will result in greater acceptability to patient and health care practitioners and provide resultant improvements in adherence and clinical outcomes;

b. Develop formulation technologies and associated scalable manufacturing processes which facilitate flexible dosing (preferably at patient level) tailored to patient needs;
c. Develop and validate nanoscale imaging and diagnostic technologies (analytical) which can be used (by patients) to measure exposure levels and clinical response to targeted therapies, to determine the required amount of medication and enable timely tracking of treatment outcomes;
d. Develop intelligent systems and devices, which are able to track adherence and outcomes (safety and efficacy) of novel medicines remotely and facilitate the customization of interventions such as the release of the therapeutic agent in response to biological and physiological stimuli;
e. Develop new, modular and flexible manufacturing platform(s) capable of handling both large and small-volume, stable and unstable medicines, reducing operational and capital cost and allow rapid adjustment of production capacity to support the production and delivery of low volume stratified medicines and precision medicines for diverse patients' groups;
f. Induction of appropriate responses to vaccination, introducing novel vaccines, adjuvants and delivery systems.

- Develop and apply patient-centred predictive models using diverse information sources to better understand differences between patients in clinical trials and patients in "real" life, to better predict adherence and probable outcome of a specific treatment (good and bad) for a given patient given that patient's background and diagnosis so that patients, providers and other key stakeholders can make informed decisions with regards potential benefits and risks of different treatment regimens to patients and healthcare practitioners;
- Develop methodologies to evaluate the holistic impact of new medicines and vaccines as well as their accompanying support systems on direct and indirect healthcare and societal costs taking into account the complex interaction between pharmacological, psychological and psychosocial factors;
- Developing an efficient regulatory and HTA strategy for evaluating integrated treatment programs rather than single medicines or vaccines;

- Create the education and training materials and platforms effort to establish an adequate skill base in the industry, academia, and health agencies to implement new integrated treatment options.

In summary, a total of forty objectives were identified for the IMI2 programme, which should be considered by intended funded projects. The Call 21 for proposal was released on 3rd of March 2020.

As illustrated in Figure 10, IMI2 programme is dependent of a multi-stakeholder (medicine developer, patients, citizens, regulators, HTA agencies and healthcare providers) partnership to revolutionise the current medicine discovery and development process and continue to build Europe as a global leader in the delivery of healthcare solutions for medicines of priority to society.

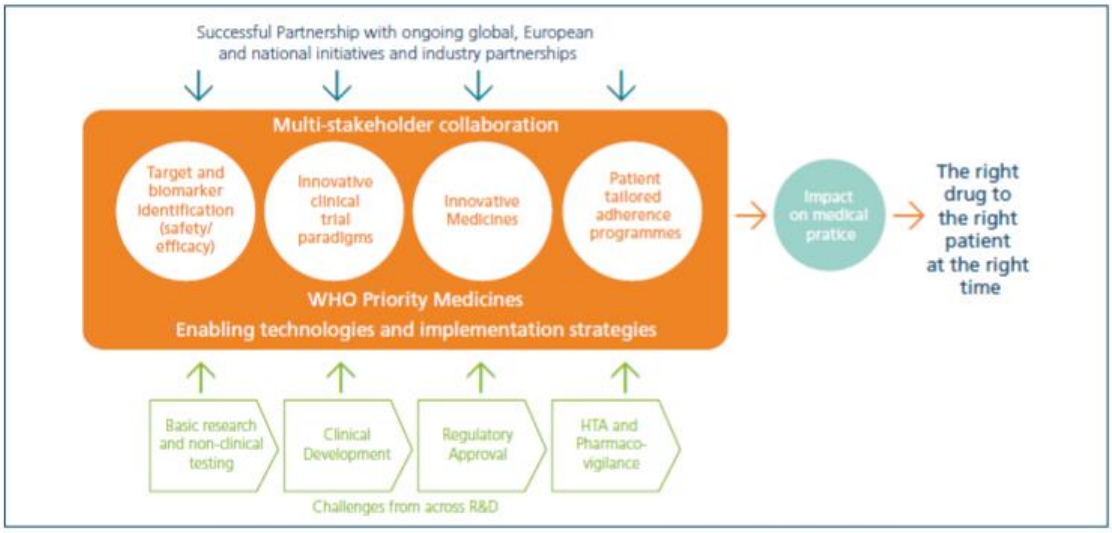

Figure 10 – The integrated vision of IMI2 programme to developed results in personalized medicine. Source: Strategic Research Agenda for Innovative Medicines Initiative 2.

As previously mentioned, both IMI1 and IMI2 programmes addressed key European health priorities defined by WHO as important areas of unmet medical need, impacting millions of citizens. The first programme focused its action on five priority diseases areas, namely Cancer, Brain disorders, Inflammatory diseases, Metabolic diseases (with a focus on diabetes) and Infectious diseases. The IMI2 programme highlighted the following twelve key health priorities, including several addressed by IMI1 programme and new large-scale programmes on Ebola, Pain, Autism, Orphan Disease, Skin Diseases, Eye diseases, Vaccines and 'Big Data for Better Outcomes' (6).

On the IMI website, the disease areas in project factsheets section are Antimicrobial Resistance, Osteoarthritis, Cardiovascular Diseases, Diabetes And Metabolic Disorders, Neurodegenerative Diseases, Psychiatric Diseases, Respiratory Diseases, Autoimmune Diseases, Ageing-Associated Diseases, Cancer, Infectious Diseases, Ebola And Related Diseases, Alzheimer's Diseases, Pain, Autism, Eye Diseases, Skin Diseases and Rare/Orphan Diseases.

Given the subject of this dissertation, the current major barriers in Diabetes will be presented in greater detail to clarify the reason why it was identified as a priority disease by WHO and is targeted for funding by IMI.

Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by a defined phenotype, including hyperglycaemia accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids and proteins, triggered by either lack of insulin secretion or decreased sensitivity of the tissues to insulin (9–11).

Due to the exponential rising of diabetes worldwide alongside its prevalence, the WHO considered this disease as the pandemic of the 21st century (10).

In 2000, over 150 million individuals were living with diabetes around the world and the projections indicated that in 15 years the prevalence of diabetes would be 250 million (1). However, the increase was higher and in 2015 the International Diabetes Federation (IDF) estimated that, globally, 415 million people had DM, which was equivalent to one in eleven adults aged 20–79 years. A time scale of 25 years projections indicated that by 2040 the prevalence was expected to rise to 642 million individuals (12), with an increasing growth in all geographical areas (Europe, North America & Caribbean, South & Central America, Africa, Middle East & North Africa, South-East Asia and Western Pacific), as presented in Figure 11. The largest numbers of people with diabetes were estimated for the WHO South-East Asia and Western Pacific Regions, accounting for approximately half the diabetes cases in the world.

Figure 11 - Estimated number of people with diabetes worldwide and per region in 2015 and 2040 (20 to 79 years). Source: Aliclic, R., Tuttle, K., 2018, Diabetes Mellitus, Hypertension: A Companion to Braunwald's Heart Disease (Third Edition), Elsevier.

The diabetes data report 2010-2045 estimated that the number of adults (20-79 years) with DM worldwide would be 463 million, 570 million and 700 million in 2019, 2030 and 2045, respectively, an increase of 51% of the cases (12). See Figure 12.

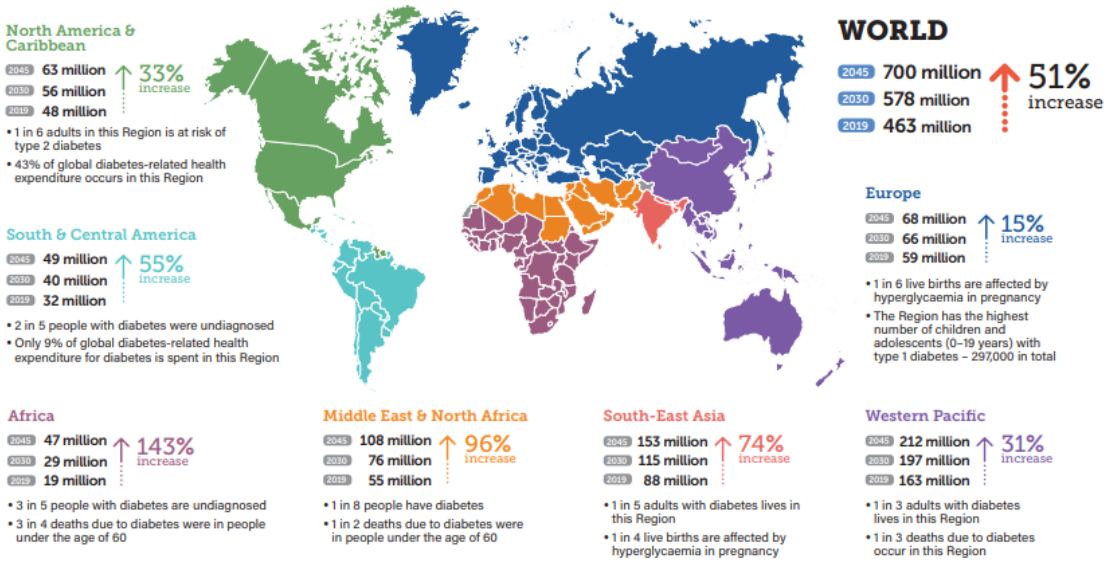

Figure 12 - Number of adults (20-79 years) with diabetes worldwide from 2019 to 2045. Source: IDF Diabetes Atlas, 9th Edition, 2019.

From a general perspective, the global prevalence of diabetes among adults aged 18 years and older has risen from 4.7% in 1980 to 8.5% in 2014 (10), the quadruple, as illustrated in Figure 13. Besides, WHO estimated that it could reach more than 20% of the world's population within the next 20 years (10,13).

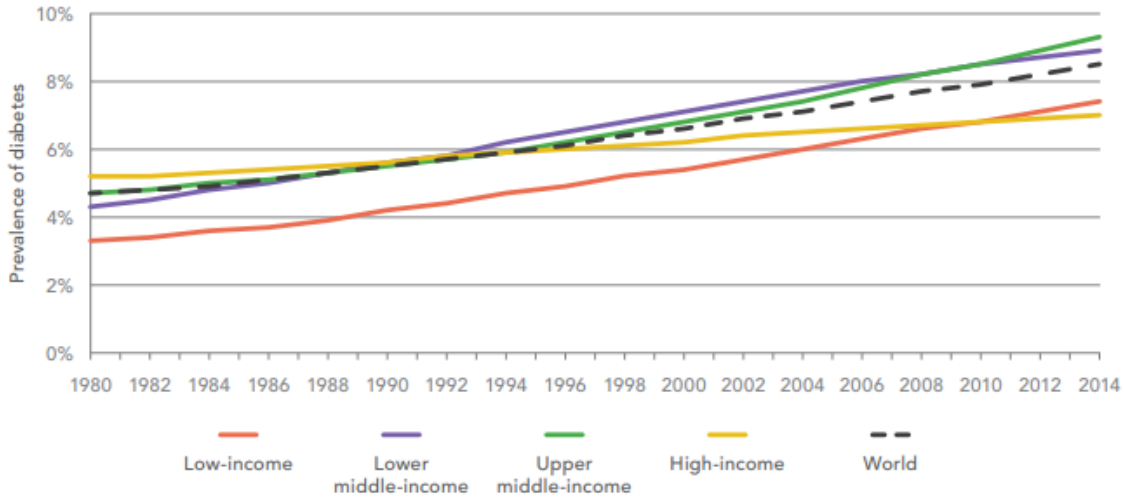

Figure 13 – The estimated prevalence of diabetes from 1980 to 2014. Source: WHO, Global Report on Diabetes - 2016.

In addition, according to the WHO, the diabetes-associated mortality rate is increasing. The worldwide estimations indicated that DM directly caused 1.5 million deaths in 2012, being the eighth leading cause of death, and 1.6 million deaths in 2016, with the classification of the seventh leading cause of death (14), as presented in Figure 14. This value represents a rise of 100.000 deaths in four years.

Moreover, in 2012 higher-than-optimal blood glucose caused an additional 2.2 million deaths, by increasing the risks of cardiovascular diseases (CVD), diabetic nephropathy (DN)/diabetic kidney disease (DKD) and other diseases. Therefore, diabetes and higher-than-optimal blood glucose together were responsible for 3.7 million deaths, many of which could be prevented (14).

Generally, almost half of diabetes and high blood glucose deaths occur in people under the age of 70 years and ≈ 80% of diabetes deaths occur in low and middle-income countries (14).

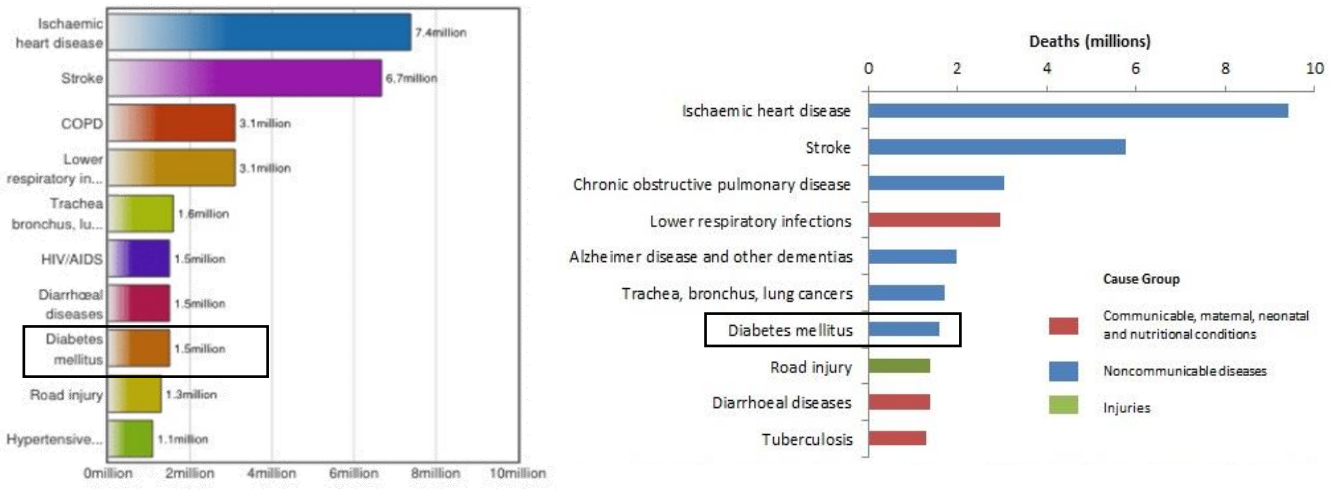

Figure 14. Top 10 global causes of deaths in 2012 (bar chart at left) and in 2016 (bar chart at right). Source: World Health Organization's Global Burden of Disease Study.

Worldwide, a majority of diabetic patients (80% to 90%) have Type 2 diabetes (T2D) and 5%–10% Type 1 diabetes (T1D), the two primary categories of DM. Since sophisticated laboratory tests are usually required to distinguish between T1D and T2D, separate global estimates of diabetes prevalence and mortality for these diseases do not exist (10). The existing approaches for diagnosis of patients with diabetes or pre-diabetes are (9):

- Random plasma test and Fasting plasma glucose (FPG) test that enable the analysis of the glucose concentration in the blood;
- Oral glucose tolerance test (OGTT) is suitable for the evaluation of the body's response to glucose;
- Evaluation of glycation of proteins since it is controlled by the concentration of glucose in the blood and by the number of reactive amino groups present in the protein that are accessible to glucose for reaction;
- Evaluation of glycated haemoglobin (HbA_{1c}), a form of haemoglobin chemically linked to glucose. This parameter serves as an indicator of the average glucose concentration and for the monitoring of blood glucose control;
- Fructosamine test allows the determination of glycated albumin because this protein also contains free amino groups. This test can be used to monitor blood glucose.

The criteria to diagnose DM are FPG ≥7.0 mmol/L (126 mg/dL), a glucose ≥11.1 mmol/L (200 mg/dL) two hours after an oral glucose challenge, or an HbA_{1c} ≥6.5%. A random plasma glucose concentration ≥11.1 mmol/L (200 mg/dL) accompanied by classic symptoms of DM (polyuria, polydipsia and weight loss) is also enough for the diagnosis of DM (9,11,15).

T1D is characterized by β-cell destruction caused mainly through autoimmune processes, which typically onsets below the age of 15 (7,9,11). The precise mechanisms underlying β-cell destruction remain relatively unknown, however, presumably, it may involve CD4+ and CD8+ T-cells and macrophages infiltrating the islets (9,11). This disease usually leads to absolute insulin deficiency, resulting in uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues, as well as to a reduction in the expression of a number of genes necessary for target tissues to respond normally to insulin, such as glucokinase in the liver and the GLUT 4 class of glucose transporters in adipose tissue, ultimately promoting major metabolic derangements (9,16). The characteristic symptoms include weight loss, polyurea, polydipsia, polyphagia, constipation fatigue, cramps, blurred vision, and candidiasis. Efforts to suppress the autoimmune process at the time of diagnosis of diabetes have largely been ineffective or only temporarily effective in slowing β-cell destruction (9). The individualized glycaemic control is achieved by diet, lifestyle, exercise and medication. Once individuals with T1D partially or completely lack endogenous insulin production, administration of basal insulin is essential for regulating glycogen breakdown, gluconeogenesis, lipolysis, and ketogenesis (9,17). The goal of T1D pharmacological management is to design and implement insulin regimens that mimic physiologic insulin secretion. Insulin replacement for meals should be appropriate for the carbohydrate intake and promote normoglycemia utilization and storage. The majority of patients with T1D should be treated with multiple daily injections of prandial (long-acting and intermediate-acting) and basal (rapid-action and regular or short-acting) insulin, or continuous subcutaneous insulin infusion (9,18). The main undesirable effect of insulin therapy is hypoglycaemia, which severity can cause brain damage or sudden cardiac death. Allergy to human insulin is unusual and insulin resistance because of antibody formation is rare (15). Moreover, T1D reduces life expectancy by about 20 years (19).

Regarding T2D, this disease is a heterogeneous group of disorders characterized by insulin resistance, impaired insulin secretion, excessive hepatic glucose production, and abnormal fat metabolism. It is usually associated with family history, older age (over 60 years old), obesity (≥80% of patients are obese), and lack of exercise. Besides, T2D is more common in women, especially those who are Black, Hispanic or Native American (9,11). T2D decreases life expectancy by 5-10 years (19). In terms of pathogenesis:

- Peripheral insulin resistance, triggered by obesity and/or genetic factors, results in impaired insulin-mediated glucose uptake on target tissues, incomplete suppression of hepatic glucose output and impaired triglyceride uptake by fat. In the early stages of the disorder, glucose tolerance remains near-normal, despite insulin resistance, because the pancreatic β-cells compensate by increasing insulin release (hyperinsulinemia) (9,11);
- Insulin resistance in the liver reflects the failure of hyperinsulinemia to suppress gluconeogenesis, which results in fasting hyperglycaemia and decreased glycogen storage by the liver in the postprandial state (9,11);
- Insulin resistance in adipose tissue results in lipolysis and free fatty acid flux from adipocytes is augmented, leading to increased lipid (very-low-density lipoprotein (VLDL) and triglyceride) synthesis in hepatocytes;
- Lipid storage or steatosis in the liver may lead to non-alcoholic fatty liver disease (NAFLD) and abnormal liver function tests, as well as dyslipidaemia (9,11).

Additionally, insulin resistance causes β-cell failure, which shows to be decreased at ≈50% in the long-term disease (9,11). Insulin resistance and hyperinsulinemia prompt impaired glucose tolerance (IGT). Most of the T2D patients are diagnosed because of complications or incidentally (17). The main therapies for T2D include antihyperglycemic agents and insulin, if symptoms of hyperglycaemia are present or when HbA_{1c} levels (>10% [86 mmol/mol]) or blood glucose levels (≥300 mg/dL [16.7 mmol/L]) are very high. The mechanisms of antihyperglycemic agents consist in the following primary physiological actions: decrease of hepatic glucose production (biguanides), increase of insulin secretion (sulfonylureas, meglitinides, DPP-4 inhibitors and GLP-1 receptor agonists), growth of insulin sensitivity (thiazolidinediones), and block kidney glucose reabsorption (SGLT2 inhibitors) (18).

In recent years, the incidence of T2D has increased, reflecting the rise of risk factors, such as overweight and obesity in young age groups associated with lifestyle habits. In addition, it was observed a progressive increase in the prevalence of T1D in Europe, however, the causes for this increase remain unknown (10).

There is a huge unmet medical need for pharmaceutic therapies for the prevention, treatment and cure of islet β-cell function/mass or providing long-term glucose-lowering. The complex mechanisms underlying T1D and T2D and the high heterogeneity within the diabetic population (ethnicities, ages, weights, concomitant diseases and genetic factors) result in many subpopulations within each disease classification, which drives the need for the development of more tailored treatment programmes. These programmes should not only consider the mechanistic classification of disease, but also the management of risk factors which drive disease onset (1,7,20). A truly personalized approach to managing patients with diabetes should include: 1) understanding of how clinical phenotypic variation alters response or outcome, and 2) identification of molecular signatures ('omics') that improve the ability to predict outcomes (20).

Moreover, currently available therapies are not effective enough to normalise glucose levels and lipid metabolism. Therefore, the majority of diabetic patients develop acute and chronic complications. Acute complications are a significant contributor to mortality, costs and poor quality of life. Short-term exposure to hyperglycaemia may trigger diabetes ketoacidosis in T1D and T2D, and hyperosmolar coma in T2D (11). Abnormally low blood glucose may result in seizures or loss of consciousness in T1D and T2D (10).

The long-term complications of diabetes are usually divided into two categories: microvascular and macrovascular diseases. The microvascular diseases include diabetic retinopathy (DR), DKD and diabetic neuropathy (10,11). Concerning the macrovascular complications, this set is composed by NAFLD and CVD, such as heart disease (namely cardiomyopathy) and stroke (10,11). It is crucial to highlight that:

- DR is a major cause of blindness and occurs as a result of long- term accumulated damage to the small blood vessels in the retina. Retinopathy rates are higher among people with T1D. About 10% of patients with more than 15 years of DM develop severe visual impairment (10);

- Diabetic neuropathy corresponds to damage to the nerves as a result of diabetes and affects up to 50% of patients. The usual symptoms include tingling, pain, numbness, or weakness in the feet and hands. In addition, reduced blood flow combined with diabetic neuropathy prompt foot ulcers, infection and the eventual need for limb amputation (10);
- Diabetes is responsible for 12–55% of cases of DKD. The incidence of DKD is up to 10 times higher in adults with diabetes than those without. Additionally, DKD is the cause of 10-20% of diabetes-related deaths (10);
- Adults with diabetes historically have a two- or three-times higher rate of CVD than adults without diabetes. It occurs in T1D and T2D and is the cause of 50% of people with diabetes deaths (10).

DM and its complications cause not only human suffering, but they also represent a major economic burden on the global healthcare system and the wider global economy (7). This impact is measured through the consideration of direct medical costs, indirect costs, and the effect on nations' gross domestic product (GDP) (10).

Direct medical costs englobe hospital inpatient and outpatient care, medications and medical supplies, such as injection devices and self-monitoring consumables, as well as long-term care (10). The worldwide estimated annual costs of diabetes have been increasing (Figure 15), growing from $232 billion spent in 2007, to $727 billion in 2017 for adults aged 20–79 years (21). The IDF estimations for 2019 indicated a raise in 4.5%, with a total cost of $760 billion (21).

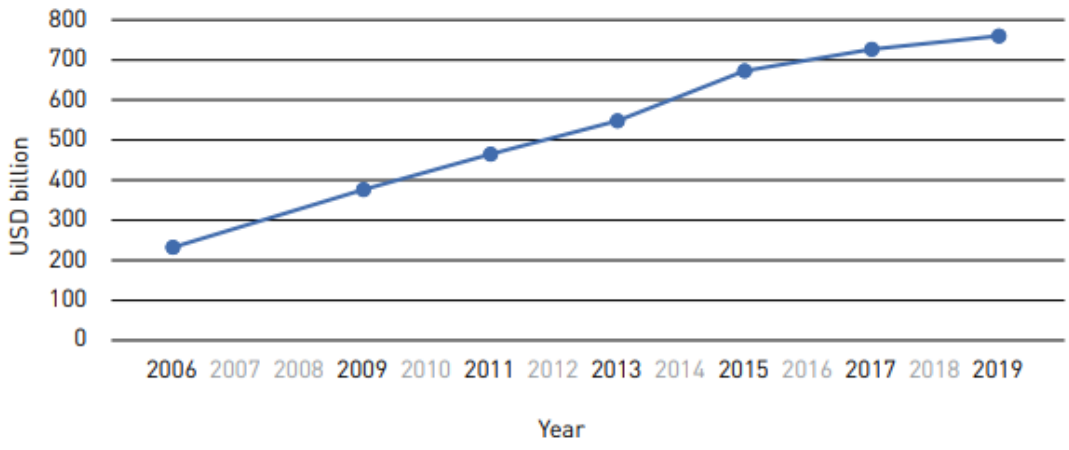

Figure 15 – Total diabetes-related direct medical expenditure for adults (20–79 years). Source: IDF DIABETES ATLAS, Ninth Edition, 2019.

Moreover, the future projection for 2030 and 2045 forecast a total expenditure of $825 billion and $845 billion, respectively (21), as presented in Figure 16. Based on these data, the direct cost will exhibit an increase of 8.6% from 2019 to 2030 and 11.2% from 2019 to 2045 (21). Low- and middle- income countries will carry a larger proportion of this future global healthcare expenditure burden than high-income countries (10).

Regarding complications of diabetes, these diseases are associated with more than 10% of healthcare costs in Europe (10,22).

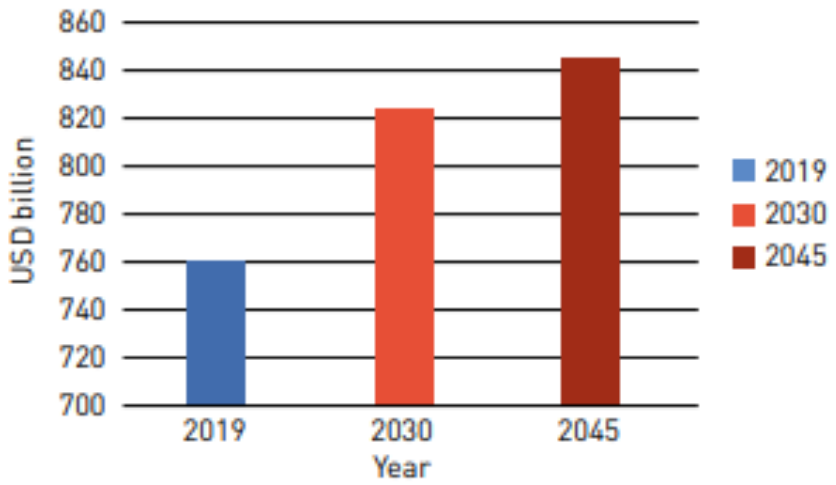

Figure 16 - Total diabetes-related direct medical expenditure projection for adults (20–79 years) in 2019, 2030 and 2045. Source: IDF DIABETES ATLAS, Ninth edition, 2019.

The indirect costs of diabetes consist of labour-force drop out, mortality, absenteeism, and presenteeism (21). Of these, the first two dominate the global picture with 48.5% and 45.5% contributions, respectively, as well as in high-income countries with 59.2% and 35.5%, respectively (21). Mortality contributes to 63.6% of indirect costs in middle-income countries and 90.6% in low-income countries (21). Absenteeism and presenteeism together contribute 6% globally and less than 3% in low-income countries (21). Overall, it was estimated that indirect costs represent 34.7% of the total global estimate of the costs of diabetes (21).

In terms of the effect on nations' GDP, WHO data indicate that worldwide from 2011 to 2030, losses in this monetary measure will total $1.7 trillion, comprising $900

billion for high-income countries and $800 billion for low- and middle-income countries (10).

In order to slow the increasing prevalence of diabetes, IMI sets its focus on projects aimed at understanding T1D and T2D, developing new precision medicines, identifying better patient-focused outcome measures for diagnosis, treatment selection and prognosis of T1D, T2D and complications of diabetes, and promoting better lifestyle management and adherence to prescribed medicines (1,7). Once more, to be successful in driving a significant improvement to current healthcare practices, it will require the collaboration between industry medical professionals, regulators, citizens and patient organizations (1,7).

IMI-Projects for Diabetes

In the Diabetes & Metabolic disorders field six projects were funded under the IMI1 programme (23), namely: Surrogate markers for micro- and macro-vascular hard endpoints for innovative diabetes tools (SUMMIT), Improving beta-cell function and identification of diagnostic biomarkers for treatment monitoring in diabetes (IMIDIA), Diabetes research on patient stratification (DIRECT), Stem cells for biological assays of novel medicines and predictive toxicology (StemBANCC), European Medical Information Framework (EMIF) and European Bank for induced pluripotent Stem Cells (EBiSC). The project logos are pictured in Figure 17.

SUMMIT was funded on call 1 and had a duration of almost six years- from 01/11/2009 to 31/10/2015 (23). The total project budget was €32.6 million (23). Its major aims were 1) identify and characterize novel genetic markers and soluble biomarkers associated to diabetic complications: diabetic nephropathy, diabetic retinopathy, lower extremity arterial disease and cardiovascular disease; and 2) improve and develop novel knowledge, imaging techniques for monitoring progression of diabetic vascular complications, and novel animal and *in silico* models for better prediction of micro- and macro-vascular diabetic complications (24).

IMIDIA was also funded on call 1, with a total budget of €25.9 million and had a duration of five years and seven months (23). This project focused its efforts on the following goals: 1) deliver novel tools for the study of human beta-cell development,

function and survival, their modulation by potential therapeutic compounds, and for *in vivo* beta-cell imaging; 2) deliver biomarkers for the diagnosis and prognosis of beta-cell failure and for monitoring diabetes progression and treatment; and 3) deliver knowledge on novel molecular pathways and sites that control β-cell life and death as well as mass and function (25).

DIRECT was funded on call 3 and received a budget of €43.1 million for a seven years project (since 01/02/2012 to 31/07/2019) (23). This project aimed at identifying the subtypes of type 2 diabetes patients, identifying and validation of biomarkers associated with different subtypes of type 2 diabetes and different rates of disease progression, and determination of the most appropriate treatments for each subtype of type 2 diabetes patients (26).

StemBANCC was funded on call 4 and was carried out from 01/10/2012 to 31/03/2018 (23). The total contribution was €55 million (23). The key-objectives established were to generate high quality human-induced pluripotent stem (iPS) cell lines to study a range of chronic diseases (peripheral neuropathies, neurodegenerative disorders, neurodysfunctional disorders, diabetes), to test for medicine efficacy and safety, and to characterize iPS cell lines in terms of their genetic, protein, and metabolic profiles (27).

EMIF was also funded on call 4, with a total budget of €55.8 million and a duration of five years and five months (01/01/2013 - 30/06/2018) (23). The major goals of this project were to develop a common information framework of patient-level data that will link up and facilitate access to diverse medical and research data source, to identify predictors of metabolic complications in obesity, and to identify predictors of Alzheimer´s disease in the pre-clinical and prodromal phase (28).

EBiSC was funded on call 8, receiving a budget of €34.1 million (23). This project aimed at establishing an European iPS cell bank to be a quality resource for the characterisation, storage and distribution of high-quality iPS cells (29). Its duration was almost of three years (01/01/2014 to 31/12/2017), however, after its conclusion, a second project phase (EBiSC2) was launched on 1st March 2019 incorporating key members of the first project phase in order to distribute disease-relevant and high-quality iPSCs, along with comprehensive datasets (23,29).

Figure 17 - Logo of the projects funded by IMI1 programme for diabetes.

In IMI2 programme, until October of 2019, seven projects were supported in the area of Diabetes & metabolic disorders (23). These projects were: Translational approaches to disease modifying therapy of type 1 diabetes: an innovative approach towards understanding and arresting type 1 diabetes (INNODIA), Assessing risk and progression of prediabetes and type 2 diabetes to enable disease modification (RHAPSODY), Biomarker enterprise to attack DKD (BEAT-DKD), Liver Investigation: Testing Marker Utility in Steatohepatitis (LITMUS), Hypoglycaemia - redefining solutions for better lives (Hypo-RESOLVE), Investigating mechanisms and models predictive of accessibility of therapeutics into the brain (IM2PACT), and Cardiomyopathy in type 2 diabetes mellitus (CARDIATEAM). The project logos are presented in Figure 18.

INNODIA was funded on call 1, with a total contribution of €35.6 million, and runs from 01/11/2015 to 31/10/2022 (23). This project aims to advance the understanding of type 1 diabetes and address the lack of tools and technologies that will allow clinicians to predict, evaluate and prevent the onset and progression of type 1 diabetes. Besides that, it aims to perform clinical intervention studies leading to novel therapies for preventing and curing type 1 diabetes (30).

RHAPSODY was funded on call 3, receiving a budget of €18.5 million for a project with a start date at 01/04/2016 and end date at 31/03/2020 (23). This intends to understand the factors that drive the progression of pre-diabetes to diabetes, and the

deterioration of the condition of people with diabetes, as well as to develop novel biomarkers to refine diagnosis leading to better patient stratification, promote prevention, and support innovative medicine discovery for personalized management of type 2 diabetes (31).

BEAT- DKD was funded on call 5 with a budget of €18.5 million (23). It was conducted from 01/09/2016 until 31/08/2021 (23). Its objectives are providing a holistic systems medicine view of the pathogenesis of DKD with the intention to identify targetable mechanisms and pathways underlying initiation and progression of DKD, applying a novel sub-classification of diabetes. Moreover, this project proposes to identify and validate biomarkers of disease progression and treatment responses representing the first steps towards precision medicine in the management of DKD (32).

LITMUS was funded on call 9 and runs from 01/11/2017 until 31/10/2022 (23). The total contribution was €46.5 million (23). The overarching aims of LITMUS are 1) develop, robustly validate and advance towards regulatory qualification biomarkers that diagnose, risk stratify and/or monitor NAFLD and Non-alcoholic Steatohepatitis (NASH) progression and fibrosis stage; and 2) develop and validate imaging techniques that will allow doctors and researchers to rapidly and easily diagnose the severity of patients' disease and monitor changes in patients' livers (33).

Hypo-RESOLVE was funded on call 10 with €26.8 million and has a duration of forty-eight months (01/05/2018 - 30/04/2022) (23). This project aims to provide researchers and clinicians with more validated data about the condition by creating a sustainable clinical database, conducting studies to better understand the underlying mechanisms of hypoglycaemia, conducting a series of statistical analyses to define predictors and consequences of hypoglycaemia, and calculating the financial cost in European countries (34).

IMI2PACT was funded on call 12 and goes from 01/01/2019 until 31/12/2023 (23). The received budget was €17.4 million (23). The main goals of this project are to advance the understanding of the blood-brain barrier (BBB) to facilitate the development of more effective treatments for a range of neurological and metabolic disorders, develop better models of the BBB so that researchers can study it more easily, investigate the biology of the BBB in both health and disease, and the transport routes across it, and develop innovative systems capable of delivering medicines to the brain (35).

CARDIATEAM was funded on call 13 with €12.9 million and has a duration of five years (01/03/2019 - 29/02/2024) (23). The CARDIATEAM project aims to determine how type 2 diabetes represents a central mechanism contributing to the pathogenesis and progression of diabetic cardiomyopathy, as well as how distinct diabetic cardiomyopathy is from other forms of heart failure (36).

Figure 18 - Logo of the projects funded by IMI2 programme for diabetes.

A more detailed description of the above-mentioned IMI1 and IMI2 projects for diabetes can be found in Appendix 1.

Objectives

The core objectives of this dissertation are:

- Present the key-achievements of each IMI-funded project for Diabetes;
- Integrate project results into Target & Biomarker identification axis;
- Integrate project results into Innovative clinical Trials Paradigms axis;
- Integrate project results into Innovative Medicines axis;
- Integrate project results into Patient tailored adherence programmes axis;
- Systematize the main biomarkers and genetic markers developed for diabetes mellitus, type 1 diabetes, type 2 diabetes and complications of diabetes;
- Identify the models developed on patient stratification for type 1 and type 2 diabetes;
- Indicate the new potential treatment targets as well as the novel therapeutic agents developed for type 1 diabetes, type 2 diabetes and complications of diabetes;
- Identify new diagnostic technologies for diabetes mellitus and complications of diabetes;
- Propose an integrated approach for the inclusion of health outcomes in order to slow the increasing prevalence of diabetes.

Methods

In this dissertation, thirteen projects presented in the Diabetes & Metabolic Disorders Area displayed on the Innovative Medicines Initiative (IMI) were considered. The methodology applied was a literature review.

The data sources used in this review were the IMI website, the official project websites, contact with the project coordinators and co-coordinators, and the CORDIS (The Community Research and Development Information Service) database. From IMI website it was collected the project's start and end date, the grant agreement number, the contributions, and the coordinators and co-coordinators' e-mail addresses. The aim of each project was retrieved from its official website. The sources of the publications were the project's official website, CORDIS database and the contact via e-mail with the coordinators and co-coordinators (Table 2).

The contacts via e-mail with the coordinators and co-coordinators were conducted twice: January of 2019 and recall in February of 2019. This step was performed for all projects, with the exception of IMI2PACT and CARDIATEAM as these were funded by IMI later on.

For SUMMIT's project, a total of 98 citations were screened, 52 from the SUMMIT's website and 46 from the Cordis database. A total of 67 references were excluded: (i) duplicates – 29, (ii) book chapters – 2, (iii) not access to the full text – 7, (iv) the publication's objective was not related to diabetes mellitus or its complications – 9, and (v) the publication's achievements did not allow to induce a scientific advance in Diabetes field – 20. For this project, a total of 31 articles were included.

For IMIDIA's project, a total of 29 citations were screened, 13 from the IMIDIA's website and 12 from Cordis database. A total of 11 references were excluded: (i) duplicates – 5, (ii) book chapters – 1, (iii) the publication's objective was not related with diabetes mellitus or its complications – 3, and (iv) the publication's achievements did not allow to induce a scientific advance in Diabetes field – 2. For this project, a total of 18 articles were included.

For DIRECT's project, a total of 25 citations were screened on the list of publications sent by the project coordinator Hartmut Ruetten. This list can be found in Appendix 2. A total of 9 references were excluded: (i) duplicates – 1, (ii) not access to

the full text – 2, and (iii) the publication's achievements did not allow to induce a scientific advance in Diabetes field – 6. For this project, a total of 16 articles were included.

For StemBANCC's project, a total of 122 citations were screened, 91 from the StemBANCC's website and 31 from Cordis database. A total of 103 references were excluded: (i) duplicates – 30, (ii) book chapters – 1, (iii) the publication's objective was not related to diabetes mellitus or its complications – 71, and (iv) the publication's achievements did not allow to induce a scientific advance in Diabetes field – 1. For this project, a total of 19 articles were included.

For EMIF's project, a total of 165 citations were screened, all from the EMIF's website. A total of 136 references were excluded: (i) duplicates – 1, (ii) article's exclusion criterion was the presence of diabetes – 1, (iii) the publication's objective was not related with diabetes mellitus or its complications – 120, and (iv) the publication's achievements did not allow to induce a scientific advance in Diabetes field - 14. For this project, a total of 29 articles were included.

For EBiSC's project, a total of 15 citations were screened, 6 from the EBiSC's website and 9 from Cordis database. A total of 14 references were excluded: (i) the publication's objective was not related to diabetes mellitus or its complications – 14. For this project, a total of 1 article was included.

For INNODIA's project, a total of 79 citations were screened, 47 from the INNODIA's website and 32 from Cordis database. A total of 34 references were excluded: (i) duplicates – 32, (ii) not access to the full text – 1, and (iii) the publication's achievements did not allow to induce a scientific advance in Diabetes field – 11. For this project, a total of 35 articles were included.

For RHAPSODY's project, a total of 39 citations were screened, 20 from the RHAPSODY's website and 19 from Cordis database. A total of 28 references were excluded: (i) duplicates – 21, (ii) book chapters – 1, and (iii) the publication's achievements did not allow to induce a scientific advance in Diabetes field – 6. For this project, a total of 11 articles were included.

For BEAT-DKD's project, a total of 90 citations were screened, 52 from the BEAT-DKD's website and 38 from Cordis database. A total of 65 references were

excluded: (i) duplicates – 40, (ii) the publication's objective was not related to diabetes mellitus or its complications – 10, and (iii) the publication's achievements did not allow to induce a scientific advance in Diabetes field - 15

No results were identified in LITMUS, Hypo-RESOLVE, IMI2PACT, and CARDIATEAM projects.

The search and screening processes are summarized in Figure 19.

Table 2 - Summary of sources and publications included in this dissertation.

Projects	**Articles obtained by contact via Email**	**Period of time (Month / Year)**	**Publications retrieved from the project's website**	**Period of time (Month / Year)**	**Articles collected on Cordis database**	**Period of time (Month / Year)**
SUMMIT	-	01/19 - 02/19	52	02/19	42	02/19
IMIDIA	-	01/19 - 02/19	13	02/19	16	02/19
DIRECT	25	01/19 - 02/19	NA	-	NA	-
StemBANCC	-	01/19 - 02/19	91	05/19 – 06/19	31	05/19 – 06/19
EMIF	-	01/19 - 02/19	47	06/19	0	06/19
EBiSC	-	01/19 - 02/19	6	06/19	9	06/19
INNODIA	-	01/19 - 02/19	47	07/19	32	07/19
RHAPSODY	-	01/19 - 02/19	20	08/19	19	08/19
BEAT-DKD	-	01/19 - 02/19	52	08/19	38	08/19

LITMUS	-	01/19 - 02/19	0	08/19	0	08/19
Hypo-RESOLVE	0	01/19 - 02/19	0	08/19	0	08/19
IMI2PACT	-	-	0	08/19	0	08/19
CARDIATEAM	-	-	0	10/19	0	10/19

NA – Not applicable

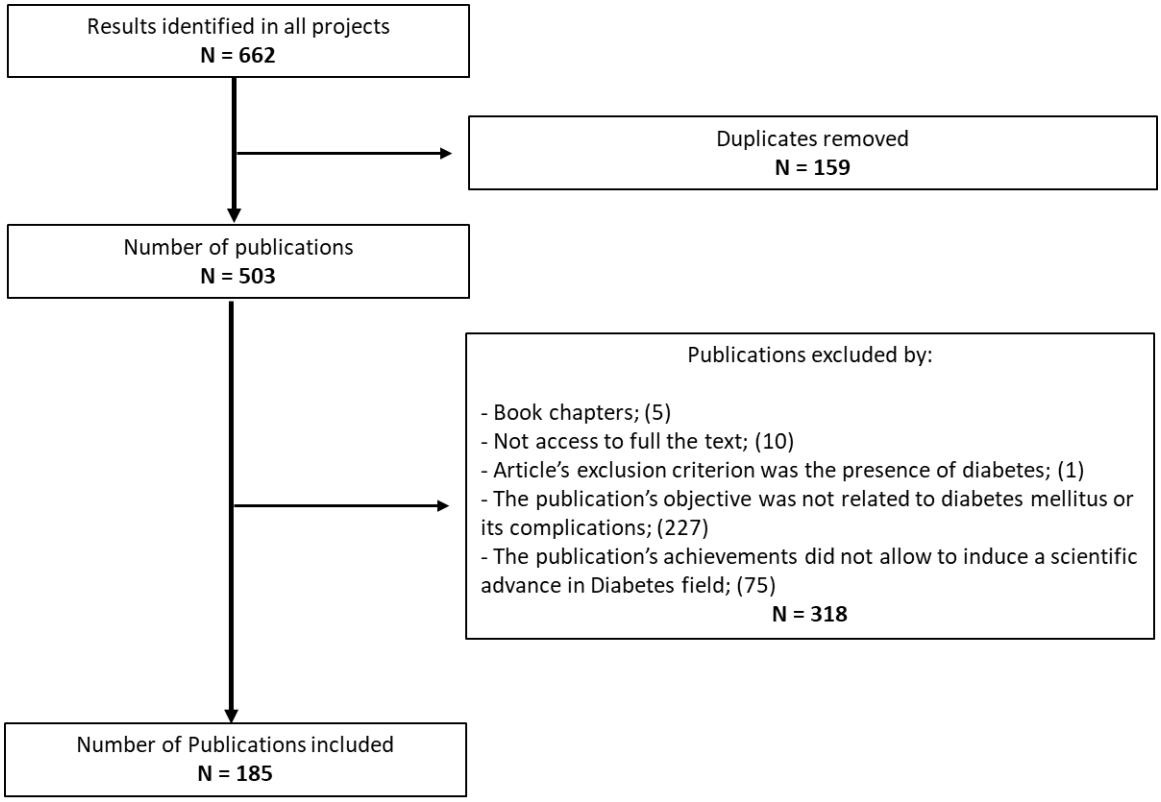

Figure 19 - Flowchart of literature search.

Discussion

Main Results of IMI-funded projects

For diabetes a total of thirteen projects were funded, however at the time of the research, only nine projects had scientific publications. The four projects without results were LITMUS, Hypo-RESOLVE, IMI2PACT and CARDIATEAM.

The results gathered in the literature review were integrated into the axes presented by the IMI2 programme, namely target & biomarker identification, innovative clinical trials paradigms, innovative medicines, and patient-tailored adherence programmes, as illustrated in Figure 20. The data collected was organized according to the goals established by each axis, previously presented in the Introduction. Besides, a summary table with a detailed description of the outcomes is available in Appendix 3.

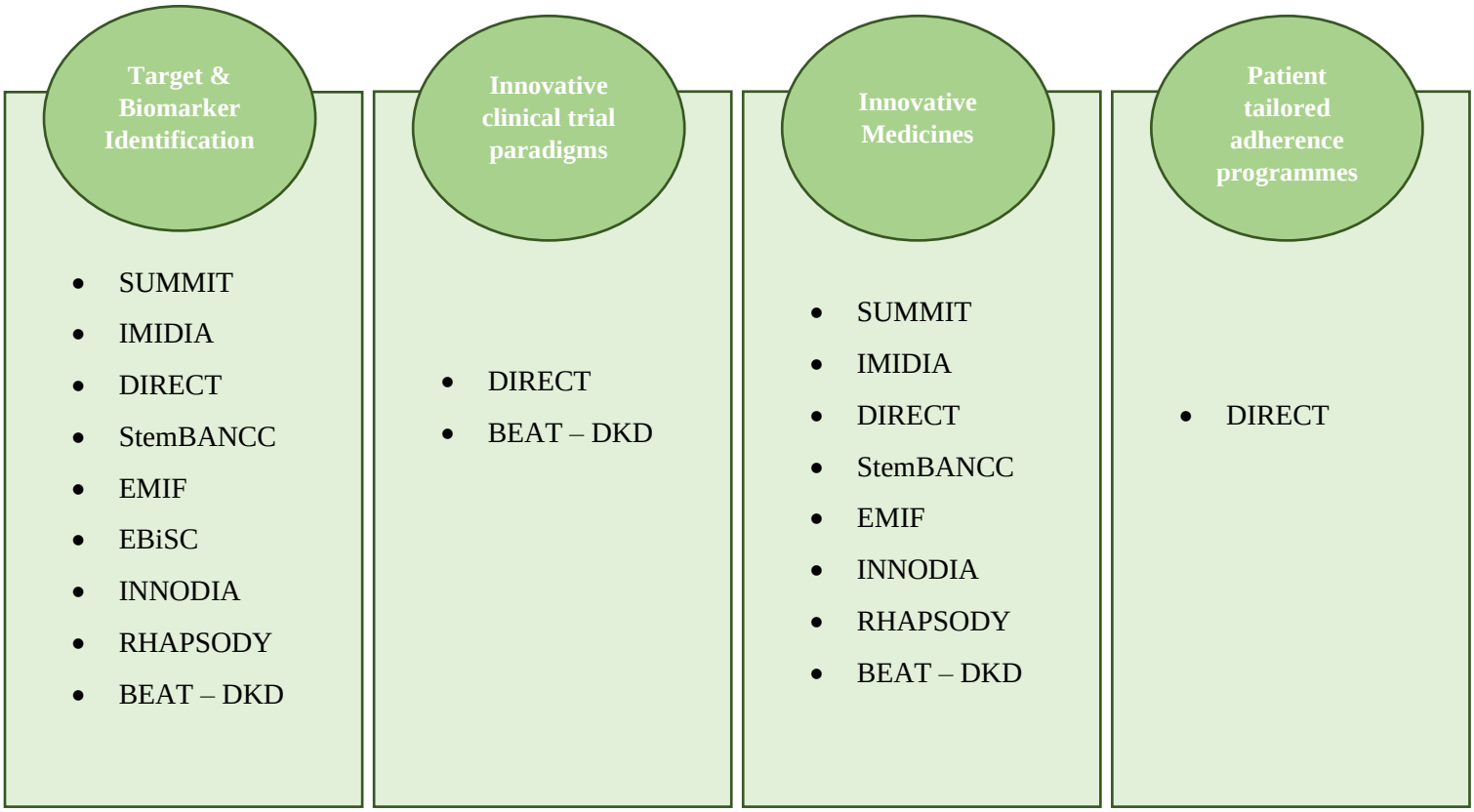

Figure 20 – Projects' distribution according to their outcomes and the axes of the IMI2 programme.

Axis I: Target & Biomarker Identification

In "Target & Biomarker Identification" axis, five subgroups were identified (Figure 21), more precisely: 1) identify and validate biological markers, tools and assays, 2) determinants of inter-individual variability, 3) understand the molecular mechanisms underlying the disease, 4) develop a platform of pre-clinical assays, and 5) develop systems models.

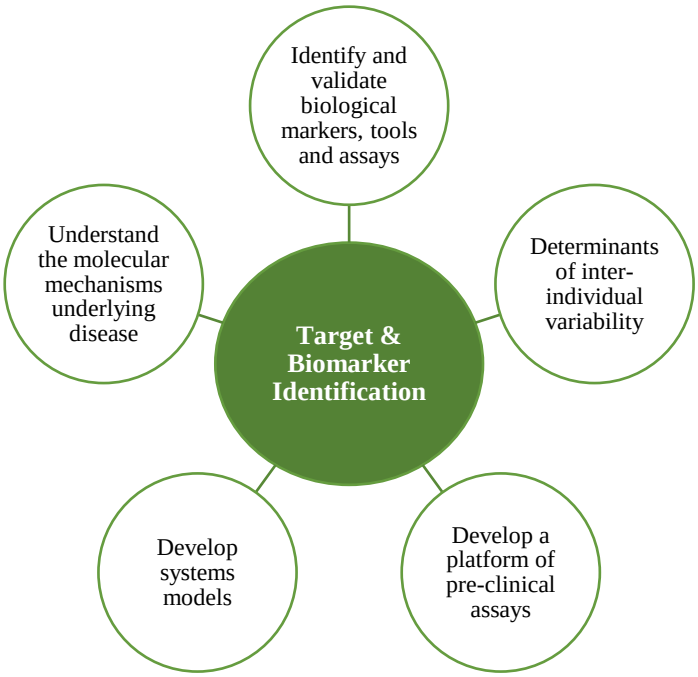

Figure 21 - Schematization of the "Target & Biomarker Identification" axis in five major subgroups according to the suitability of the data collected from IMI-funding projects in the defined goals presented by the IMI programme.

Identify and validate biological markers, tools and assays

The projects that presented novel soluble biomarkers were SUMMIT, IMIDIA, DIRECT, EMIF, INNODIA, RHAPSODY, and BEAT-DKD.

For type 1 diabetes, INNODIA proposed novel β-cell biomarkers, more precisely DPP6, FXYD2γa, PDL1, the antibodies GAD, IA-2 and ZnT8 (37–40), and autoantigens, such as ER chaperone GRP78, CHGA, PTPRN, GAD2, SLC30A8, IAPP, MTIF3, PPIL2 and MLH1 (41–43). In addition, some biological factors were associated with protection

against T1D by their pro-survival role in human β-cells, among which HLA-DQ6, complement system C3 and Bcl2-A1 protein (37,44–47).

Aimed at type 2 diabetes, some of the biomarkers proposed were high free fatty acids levels (IMIDIA) (48), valine to phosphatidylcholine acyl-alkyl C32:2 metabolite (DIRECT) (49), small-gamma-glutamyl transpeptidase, plasma mannose, plasma metabolites, SIRT1, branched-chain amino acids, 3-Hydroxyisobutyric acid, tyrosine, alanine transaminase, aspartate aminotransferase, C-reactive protein, adiponectin and CCL18 serum levels (EMIF) (50–59). Furthermore, EMIF suggested two histological markers for T2D abnormalities, more precisely visceral adipose tissue and subcutaneous adipose tissue adipocytes (60), and SUMMIT disclosed the lack of evidence of lower adiponectin levels as biomarkers to identify individuals at risk of the disease (61).

SUMMIT validated biomarkers related to the following diabetic complications: i) DKD, such as free and total desmosines since it exhibited utility for disease progression, patient stratification and medicine efficacy (62), ii) CVD, with emphasis on matrix metalloproteinases 7 and 12 since they were associated with vascular complications in T2D, and iii) DR, mainly pathogenic factors, more specifically the vascular endothelial growth factor (VEGFA), nuclear factor of activated T-cells (NFAT), caspase-3 and advanced glycation end products. (63–65).

BEAT-DKD validated some specific biomarkers related to the initiation of DKD in patients with type 2 diabetes, such as urinary extracellular vesicles, KIM1 and FGF23 (66–69). Besides, this project identified an early prognostic marker for DKD rapid progression, namely the urinary epidermal growth factor (70), a set of thirty-three compounds with the potential of reversing the expression signature of renal age-associated DKD prognosis genes (71), a biomarker of treatment choice, i.e. the NT-proBNP (72), and thirty-eight neuroprotective markers of DKD, among which HMOX1, NFE2L2 transcription factor and SIRT1 (73,74).

Additional new biomarkers were proposed by RHAPSODY for pancreatic β-cells, more especially transcription factor MondoA, MAF and PAX6 (75,76), as well as for β-cell dedifferentiation, namely fibroblast growth factor 1, fibroblast growth factor 2, SOX9, NEUROG3 and gastrin (76). RHAPSODY also exposed some associations between biological proteins and hyperglycaemia, such as SLC2A2, FFAR4, TMEM37, TMEM27 and ALDOB (77).

Regarding novel tools, we subdivided the results into five categories, according to their purpose.

The first one comprises tools related to diabetes, highlighting the manganese-enhanced high field magnetic resonance imaging (MEHFMRI) developed by IMIDIA for diagnostic and monitoring diabetes progression (78–83), the glutamic acid decarboxylase autoantibodies (GADA) test, a new tool proposed by DIRECT for prediction of diabetes progression (84), and a machine learning developed by RHAPSODY to predict the glucose tolerance status by twenty-two baseline variables obtained at fasting blood acquisition (85). Moreover, IMDIA developed a glucose nanosensor for intracellular glucose imaging in mammalian cells, the FLII12Pglu-700μ-δ6, intended to promote real-time analysis of glucose-induced insulin secretion in pancreatic beta cells and understand defects in diabetic conditions (82).

The second category includes tools developed by INNODIA specifically for T1D since it was verified that less than half paediatric and adolescent patients use continuous glucose monitoring (CGM) device (86). This set includes the Dried Blood Spots (DBS) method intended to measure C-peptide in children and adolescents recently diagnosed and track β-cell function over the time at T1D risk patients (87), the novel β-cell function algorithm to predict the pathway of β-cell function in recent-onset type 1 diabetes (88), and a paramagnetic contrast agent, Gd-DOTA-P88, which is based on a peptide (P88) that targets FXYD2γa, a β-cell biomarker, enabling its screening (38).

The third category comprehends tools conceived for T2D, more specifically the triple test, a non-invasive tool proposed by EMIF to predict the amount of liver fat in the follow-up of T2D patients with insulin treatment (89,90).

The fourth category comprises tools developed by SUMMIT for diabetic complications, emphasizing the Ultrasound Plaque Structure Analysis (UPSA)-system, a non-invasive, low cost and safe method that enables the assessment of atherosclerosis plaques structure, the detection of patients at high risk of a heart attack or stroke, and the monitoring of individual's response to interventions (91). Also, this set contains the 18F-fluoromethylcholine (18F-FMCH), a potential tracer for the evaluation of atherosclerotic vascular inflammation in diabetes (92), and the Optical Coherence Tomography (OCT), a large dataset composed by macular thickness measurements to improve prediction of diabetic retinopathy (65).

Lastly, the fifth category encompasses the new tools used on genetic research, mainly prediction tools. It includes those validated for SNP selection and multivariate analysis, whose goal was the identification of common and rare variants associated with increased people's risk of developing diabetes and its complications and with disease's progression, namely the Hierarchical Naïve Bayes (HNB), the Binary Outcome Stochastic Search (BOSS), the 'Bag of Naïve Bays' (BoNB) and the Algorithm based on a Bivariate Cumulative Statistic (ABACUS) validated and published by SUMMIT (93–97). Moreover, StemBANCC developed the T2D phenotypic-linkage network (T2D-PLN), which intends to integrate functional genomics data type and provide an accurate prediction of gene influence on T2D risk (98) and upgraded 'Genome editing' technologies used on genetic research to generate isogenic control lines, highlighting the CRISPR-Cas9 (99).

Determinants of inter-individual variability

The second subgroup concerns the knowledge developed on matters of a better understanding of molecular determinants of inter-individual variability to treatment efficacy and safety according to factors such as age, weight, sex, ethnicity, gut microbiota composition, genotype, and lifestyle.

A significant number of genetic markers were discovered for diabetes mellitus and its complications by SUMMIT, IMIDIA, DIRECT, EMIF, INNODIA and RHAPSODY. Since several genetic markers were discovered and we had no information about their frequency in the population, we decided not to present an example. Nevertheless, they are listed by project in Appendix 3.

In addition, the increase of diabetes mellitus' risk was associated with three SNPs in three metabolites (glycine, serine, betaine) and one in the glycine-to-serine ratio (100), all four identified by SUMMIT. According to EMIF, this risk was also linked with other four mutations and one DNA CpG methylation (50,59,101–104). Besides, RHAPSODY suggested specific loss-of-function mutations as a monogenic cause of early-onset diabetes during adolescence or young adulthood (105) and the existence of a gene (PAQR7) inversely associated with HbA_{1c} levels (106).

For T1D, INNODIA identified thirteen gene markers associated with the development risk of this disease (44,45,107–110).

Regarding T2D, IMIDIA proposed twenty-three genetic markers associated to predisposition as well as its development and progression (80,83,111–114), RHAPSODY identified new SNPs associated with its increased susceptibility (77,106) and DIRECT discovered a set of twenty-one alleles associated with first-phase insulin secretion that influence risk of disease and sixty-five body mass index (BMI)-associated loci linked with this disease (115–119). Besides, DIRECT evidenced that genetic variations associated with T2D predisposition were different from those that caused its progression (84).

Concerning diabetic complications, SUMMIT proposed twenty-one SNPs with individual increased risk of DKD, one SNP with DKD extension, a sex-specific genetic variant that underlies women protection against end-stage renal disease in type 1 diabetes, and discarded some potential false-positive genetic markers associated with DKD (64,65,127,95,120–126). In addition, SUMMIT identified three known coronary artery calcification SNPs associated with the development of CVD complications in patients with T2D (96). Furthermore, it was identified by DIRECT and EMIF that four genetic variants loci nominally associated with GLP-1 stimulated insulin secretion (128) and the loss of function of two proteins (EMISCD1 and ELOVL6) led to improved insulin sensitivity (129).

Regarding the genetic findings highlighted for inter-individual variability to medicines' efficacy of metformin in T2D patients, SUMMIT discovered that the glycaemic response for the absolute reduction in HbA_{1c} diverges in 34% according to the hereditable phenotype (130). Moreover, DIRECT observed that polymorphisms in organic cation transporter 1 (OCT1) altered tolerance to this medicine and gene variations in SLC47A, ATM and NPAT modify drug-response (20,131). For sulfonylureas, DIRECT detected that patients with HNF1A mutations respond better to sulfonylureas than metformin, and cytochrome P450 2C9 (CYP2C9) *2 and *3 variation change sulphonylurea efficacy (20,131).

Moreover, obese patients with T2D and with rs7903146 in TCF7L2 gene had a significant association with a lower reduction of fasting blood glucose after Roux-en-Y Gastric Bypass surgery (132).

In aspirin-treated patients with diabetes, SUMMIT referred that the variable rate of turn-over of the medicine target represented the main mechanism of response variability (133,134).

A non-genetic inter-individual therapeutic variability factor was also identified by BEAT-DKD. This project observed that poor responders that initiated treatment with renin-angiotensin-aldosterone system (RAAS) inhibition within a type 2 diabetic patients' group, treated in primary care, had the highest risk of cardiovascular events and showed large variability in urinary albumin creatinine ratio (UACR). In cases with higher NT-proBNP levels aliskiren increased the risk of the cardio-renal endpoint and at lower NT-proBNP tertile, treatment with aliskiren tended to reduce cardio-renal risk (72,135,136).

In respect to gut composition factors, DIRECT referred that microbiome alterations induced by metformin should promote intolerance and affect efficacy (19,82).

Regarding ethnicity, EMIF showed that South Asians have a greater prevalence of type 2 diabetes than do the background populations of the countries to which they moved, and the neonates seem to be more insulin resistant than white Europeans throughout childhood. Besides, this population's faster progression of retinopathy and nephropathy suggests they might benefit from early use of pharmacologic treatments (137).

According to DIRECT, the age of diagnosis of T2D influences the disease's progression, since individuals detected at a younger age of 50 years progressed rapidly compared with those aged over 70; and HbA_{1c} at diagnosis was higher in the younger than in the older group (84). Sex showed also variability due to the fact that women were independently associated with rapid progression to the requirement of insulin treatment (138). The year of diagnosis also showed to impact the progression of T2D, since individuals diagnosed prior to 2001 had a higher rate of deterioration than individuals diagnosed in 2006 or afterwards (84).

Lastly, DIRECT observed that individuals with normal weight at diagnosis were likely to be β-cell deficient and progress rapidly to insulin requirement, and those who had more particularly tissue adipose accumulation were likely to be markedly insulin resistant (138). Similarly, higher BMI (25-30 kg/m^2) and lower HDL-cholesterol (<1 mmol/l) were associated with a higher glycaemic deterioration (84). Besides, EMIF

verified that individuals classified as metabolically unhealthy had a higher relative risk of onset type 2 diabetes compared with individuals classified as healthy independent of BMI category (139) and DIRECT discovered that maternal obesity during pregnancy raised the offspring's risk of having type 2 diabetes (140).

In addition, two models of patient stratification were proposed, one by INNODIA and another by RHAPSODY and BEAT-DKD. These models can be used towards personalised treatment of diabetes.

In respect to INNODIA's model, five groups with distinct trajectories of glycaemic control (HbA_{1c}) in children from age 8 and young adults with T1D were established. Groups differed by self-care with a lower frequency of self-monitoring of blood glucose (SMBG) in subjects with HbA_{1c} deterioration and higher insulin dose among patients with increased HbA_{1c} (141).

RHAPSODY and BEAT-DKD identified five subtypes of patients with different risk levels for certain complications associated with diabetes. Cluster 1 was characterised by early-onset disease, relatively low BMI, poor metabolic control, insulin deficiency, presence of GADA and was labelled as severe autoimmune diabetes (SAID); Cluster 2 was categorised as severe insulin-deficient diabetes (SIDD), was GADA negative, low age at onset, relatively low BMI, low insulin secretion and poor metabolic control; Cluster 3 was characterised as severe insulin-resistant diabetes (SIRD) was characterised by insulin resistance and high BMI; Cluster 4 was labelled by obesity but not by insulin resistance and was labelled as mild obesity-related diabetes (MOD), and finally, Cluster 5 was categorised as mild age-related diabetes (MARD), older patients and similar to cluster 4, only with modest metabolic derangements. Patients in group 2 ('severe insulin-deficient diabetes') were at the greatest risk of eye disease, while patients in group 3 ('severe insulin-resistant diabetes') had the highest incidence of kidney damage (142).

Understand the molecular mechanisms underlying disease

In this subgroup, we will describe the most relevant pathways proposed for β-cell development and function, type 1 diabetes, type 2 diabetes, and diabetic complications.

IMIDIA discovered some relevant pathways for β-cell apoptosis, with emphasis on the effect of pro-inflammatory cytokines (143,144) and downregulation of ELOVL2

(145). For β-cell protection/ pro-apoptotic effects, IMIDIA identified the role of connexin 36 (Cx36), overexpression of H_2O_2-inactivating catalase (146,147), monounsaturated fatty acids (MUFAs) and increase of ELOVL2 expression (145). Moreover, novel pathways were proposed for pancreatic and β-cells development, during embryogenesis in rats and humans, and information regarding the dedifferentiation and redifferentiation processes in human β-cells highlighted key-markers and their role, with outputs of RHAPSODY as well (76,148–153).

Regarding T1D, INNODIA's findings supported the role of a systemic autoimmune component in the disease development proposing novel mechanisms for β-cells function/self-destruction through secretion of ER chaperone GRP78, the action of DPP-4 and IFNα, the dysregulation of miRNAs, and the levels of CD^{57+}effector memory compartment of $CD8^+$ T-cell (42,45,154–157). Novel factors were associated with early onset of T1D, more precisely telomere length dynamics and glycaemic control (158), and age and puberty were linked with specific pathological features (159,160). Furthermore, it was verified that lifestyle interventions, especially exercise training programs, prevented, delayed or attenuated loss of functional β-cell mass in T1D, in part via activation of the transcription factor STAT3 (partially via IL-6) in β-cells and consequently ERK1/2 activation (47).

For T2D some mechanisms were proposed by DIRECT in order to mediate its progression beyond genetic factors, such as glucolipotoxicity, endoplasmic reticulum stress and oxidative stress (84). As protective pathways, EMIF observed that patients with NAFLD associated with TM6SF2 variant had a decreased risk of developing T2D (161), and RHAPSODY found that amylin and nucleobindin–1 inhibited insulin fibrillation, attenuated fibril-induced cell toxicity and acted in the control of amyloidogenesis (162).

Regarding T2D complications, SUMMIT proposed that the plaque development in patients with diabetes didn't occur due to diabetic metabolism itself but mainly because of increased cholesterol levels (163) and suggested that CVD events might occur at a lower atheroma burden in diabetes (65). Moreover, this project identified a novel phenotype form of diabetic retinopathy with alterations in retinal morphology, such as biochemical alterations, apoptosis of neuronal and vascular cells in the retina (63). It was also proposed a new pathway for this disease through key pathogenic factors such as VEGFA, NFAT, advanced glycation end products, and caspase-3 (63–65). BEAT-DKD

published novel pathways associated with the initiation and the progression of DKD: for the initiation phase, it was proposed the role of PAN-induced glomerular injury, miR-92a, GSK3, STAT3 and conditional depletion/loss of DNA maintenance methyltransferase Dnmt1 (69,164–166); and for the progression phase, it was suggested the association of podocyte detachment and key age-related mechanisms such as mTOR pathway, the p53 signalling cascade, cell cycle regulation and mechanisms like focal adhesion or insulin resistance (71,167,168). Besides, EMIF highlighted the association between T2D and the increased endometrial cancer risk as a consequence of its effect on the levels of sex hormone-binding globulin (SHBG) and free insulin-like growth factor 1 (IGF-1) (169); supported the role of diabetes as a risk factor of dementia by two potential pathways: dietary methyl-glyoxyl (MG) levels and/or downstream of SIRT1 (54); and reinforced that T2D is one of the risk factors to develop NASH, a type of NAFLD, since diabetic patients yield a 2-fold higher risk of NAFLD/NASH (170), with 29% of the patients with T2D and NAFLD developing NASH (89), and had proposed also several biological and genetic markers (51,56,89,90).

Additionally, about T1D complications, INNODIA observed that oral contraceptives were associated with higher cardiovascular risk and worse metabolic control in girls (171). Anorexia and bulimia were more prevalent in girls with T1D, although clinically less serious eating disorders were observed in boys, and attention-deficit hyperactivity disorder (ADHD) was more common in boys (159).

Develop a platform of pre-clinical assays

In the domain of "Develop a platform of pre-clinical assays", we included the *in vitro*, *ex vivo* and *in vivo* models created as well as other tools that helped to improve translation from pre-clinical testing to the clinical studies. With major emphasis to the main aim of this subgroup, three projects are highlighted, namely, StemBANCC, EBiSC and IMIDIA. StemBANCC repository was created to store iPSCs lines derived from several pathologies, including diabetes, which were made accessible to the scientific community through EBiSC and Coriell databases (148,172,173). With regard to EBiSC, this project developed an online catalogue, available at https://cells.ebisc.org/, which until August 2019 had available sixty-two iPSC lines for diabetes mellitus disease and three iPSC lines for Maturity-Onset Diabetes of the Young (MODY) disease. These sixty-two

iPSC lines derived from patients with monogenic diabetes, patients with early-onset familial type 2 diabetes, Wolfram syndrome, and patients without the variant disease (174,175). Lastly, IMIDIA established a multicentre biobank of human islets and pancreas tissues from organ donors and metabolically phenotype pancreatectomised patients and produced a new cell-line that express specific β-cell markers (79,83). These biobanks represent a crucial tool for harmonization, efficiency optimization, improvement of quality-generating data and reduction of pre-clinical studies in animals in diabetes research.

In terms of catalogues, StemBANCC published β-cell derived iPSCs (BiPSC)-specific differential open chromatin sites (Bi-DOCS) (176), INNODIA published the first catalogue of the HLA-I peptidome of human β-cells (43) and RHAPSODY published one with cis-eQTLs with relevance to T2D and HbA_{1c} levels (177).

Several protocols were established with the purpose of improving the reliability of laboratory assays. More precisely, IMIDIA issued one for islets isolation from human pancreatic tissue obtained from type 2 diabetes and non-diabetic patients (78–83), StemBANCC developed a protocol for differentiation of endocrine pancreas that enabled its epigenetic and transcriptional profiling characterization (172,178), and RHAPSODY published a new protocol for AMPK function study in mouse and human pancreatic islets (179).

Regarding pre-clinical (animal) models, IMIDIA created a model with pancreatic α-cell specific deletion of Tcf7l2, named Tcf7l2AKO mice (80) and SUMMIT developed new models to better replicate the usual complications observed in diabetic patients, more precisely DKD, CVD and DR, which presented significant advantages compared to previously described models (65).

Develop systems models

The subgroup “Develop systems models” includes two new *in silico* models generated by SUMMIT: the Dynamic Bayesian Network model for long-term simulation of clinical complications in T1D and the aspirin action on platelet and megakaryocyte cyclooxygenase-1 (65).

Moreover, BEAT-DKD proposed the following models: the *Drosophila* Nephrocyte, a complementary model system used with the purposes of reveal mechanisms of podocyte function and glomerular diseases (180); the systems biology models composed by the integration of bioinformatics tools to match the patient-specific non-invasive molecular profile with the medicine molecular profile to better prediction of patient's response for DKD´s treatment (181); and an *in-silico* analysis model developed to identify compounds reversing a set of renal age-associated genes significantly associated with DKD progression (71).

Axis II: Innovative clinical trial paradigms

Regarding "Innovative clinical trial paradigms" axis, the data on this topic fits into two of the objectives outlined by IMI, more precisely: 1) utilise innovative endpoints, trial designs, simulation and analytical approaches to devise new clinical trial paradigms and 2) develop innovative clinical endpoints, as illustrated in Figure 22.

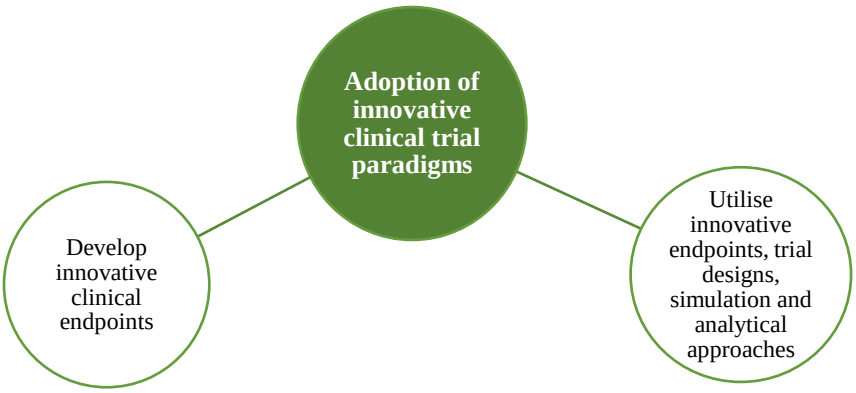

Figure 22 – In "Innovative clinical trial paradigms" axis, the data collected from IMI-funding projects apply two goals from those described in SRA.

Utilise innovative endpoints, trial designs, simulation and analytical approaches

A novel design model, the genotype-based recall (GBR), was proposed by DIRECT. This model exhibited a higher power to observe gene-drug and gene-lifestyle interactions than same-sized conventional randomized clinical trials, with exception to when the sampling frame is too small to identify minor alleles and the recalled groups are not sufficiently distinct (182).

Besides, BEAT-DKD proposed a change in the design of studies with medicines to DKD in T2D patients. Those corresponded to directly selecting patients with a positive surrogate marker response to medicine into trials and deselecting patients with a negative surrogate marker response, enabling firstly the possibility of trials to be smaller, shorter, or both, and thus potentially less expensive, and secondly the non-responders patients not be exposed to a medicine that is unlikely to benefit them (183). To help with this issue, it was proposed the concept of “basket” or “umbrella” trials, or more commonly called “platform” trials, that involves assessing the effects of multiple interventions on one or more conditions, using modern adaptive designs and statistical approaches, such as master trial protocol. The availability of multiple interventions within the platform can be used to successively select patients for clinical trials as well as enable the selection of best available therapy for each patient, paving the way for a tailored/ personalized treatment approach (183,184).

Develop innovative clinical endpoints

DIRECT developed and validated a new prediction model, DIRECT-DETECT, based on biomarkers for glycaemic deterioration before and after the onset of type 2 diabetes, which was proposed to be used in the selection process in clinical trials (185,186).

Axis III: Innovative Medicines

For “Innovative Medicines” axis, the data collected met the following goals: a) develop novel therapeutic agents and disease prevention strategies and b) implement new approaches for the development and production of biopharmaceuticals and tissue engineering, as illustrated in Figure 23. For logical and clarity reasons, we have decided to include in this topic one of the objectives set by Target & Biomarker Identification axis, more precisely “Identify new or alternative therapeutic concepts (targets) for treatment and prevention of disease”.

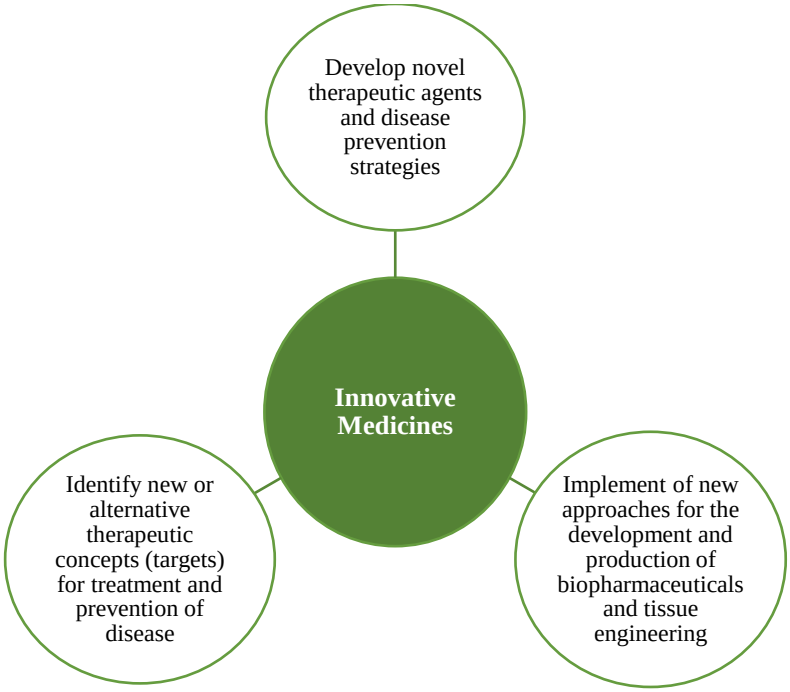

Figure 23 – Identification of the goals achieved in the "Innovative Medicines” axis by IMI-funded projects.

Identify new or alternative therapeutic concepts (targets) for treatment and prevention of disease

In the studies carried out by the IMI-funding projects, multiple proteins, genes and molecular pathways were suggested as potential therapeutic targets for diabetes.

The molecular pathways prompted by StemBANCC for the treatment of diabetes were the activation of DYRK1A and overexpression of miR-375 (148–150,187–189). In addition, for T2D, EMIF proposed the modulation of BCAA metabolism (59), and

IMIDIA suggested the activation of glucokinase, a glucose sensor in β-cells, for counteracting hyperglycaemia in individuals with type 2 diabetes (190).

Furthermore, INNODIA proposed pathways that could potentially be used to prevent or reverse the β-cell loss in type 1 diabetes, more precisely preventing GRP78 surface translocation or suppressing the GRP78 receptor signalling, increasing the expression of MANF gene, targeting IFNα and gelofusine of GLP-1R, and stimulating the activating of mTORC1 (37,107,154,155,191–193).

Concerning personalized treatments, some of the molecular pathways were proposed considering other comorbidities of T2D patients. EMIF suggested the upregulation of adipose tissue CIDEA mRNA levels in T2D patients with obesity (194). For the treatment of accelerated atherosclerosis in diabetes, SUMMIT proposed the inhibition of the Nuclear Factor of Activated T-cells signalling as a potential target (195). Furthermore, for treating glomerular disease in T2D patients the pathways proposed were reducing YAP activity, anti-miR-92a options, i.e. anti-STAT3, partial mTOR inhibition, PI3K-Akt and Raf-MEK-ERK signalling, overexpression of a positive regulator of insulin receptor or knockdown a negative regulator (PTP1B), protect podocytes from endoplasmic reticulum stress and enhancing DCXR expression (69,165,196–198).

Lastly, in terms of new/alternative therapeutic concepts, it is highlighted the therapeutic strategy hypothesis of reprogramming α-cells to produce insulin after extreme β-cell loss, proposed by IMIDIA (199), the dedifferentiation and redifferentiation of β-cells by treatment with a redifferentiation cocktail (RC) or ARX shRNA (148,189), by expression of NEUROG3 gene (150) or by controlling/decrease HIF1-α levels (187), according to StemBANCC, or as proposed by INNODIA through PolyI:C (193). Another therapeutic concept proposed, according to RHAPSODY, was the use of tRNA fragments through epitranscriptome-based therapy (200).

Develop novel therapeutic agents and disease prevention strategies

In terms of novel therapeutic agents, for type 2 diabetes, SUMMIT promoted the clinical trials of dual peroxisome proliferator-activated receptor-α/γ agonists (Aleglitazar), which was abandoned at phase 3 trial in 2013 due to safety concerns and lack of efficacy (201). Besides, SUMMIT prompt the clinical trials of RAAS inhibitors,

which demonstrated their efficacy in the delayed onset of T2D and reduce the risk of renal complications in manifested T2D (202). In addition, according to SUMMIT data low-dose aspirin for the secondary prevention of cerebro-cardiovascular events as atherothrombotic vascular complications in patients with diabetes showed to be effective, with favourable benefit/risk ratio, however, this benefit was not exhibited for primary prevention (203).

For non-diabetic population and beyond reducing the risk of developing T2D, DIRECT supported that metformin may provide cardio-metabolic benefit (204).

For the treatment of diabetic nephropathy, BEAT-DKD supported the clinical efficacy of SGLT2 inhibitors and GLP1R agonists. SGLT2 inhibitors (such as canagliflozin) slowed the progression of DKD and GLP1R agonists prevented worsening of albuminuria (205,206).

Lastly, StemBANCC established four stem cell-based replacement treatments: (i) promoting regeneration in situ by endogenous stem cells, (ii) regeneration in situ by application of exogenous stem cells, (iii) repopulating decellularized donor kidney with a patient's own stem cells, and (iv) construction of entirely new transplantable kidneys, with two broad techniques: precision engineering by positioning everything exactly (e.g 3D printing), or by supporting cells' self-organizing ability (207–209).

Implement new approaches for the development and production of biopharmaceuticals and tissue engineering

Regarding this topic, StemBANCC also focused its efforts on finding controlled large-scale production of human pluripotent stem cells (hPSCs), a crucial issue for their envisioned clinical translation in diabetes research and industrial field. Continuous peristaltic pump-based circulation technology, in a hydraulically driven bioreactor, with suspension culture of hPSCs, showed to be a potential 3D tool for large-scale production (210).

Axis IV: Patient tailored adherence programmes

Regarding the fourth axis “Patient tailored adherence programmes”, the data collected met the goal: develop and apply patient-centred predictive models. See Figure 24.

Figure 24 – Illustration of goal reached by IMI-funded projects in "Patient tailored adherence programmes” axis.

Develop and apply patient-centred predictive models

After several studies proved that individual lifestyle profile (e.g education, smoking habits, physical activity, diet and BMI) is associated with the incidence of T2D, a clear perception emerges of the need for personalized medicine (prevention, diagnosis and treatment). In consequence, DIRECT developed new screening tools, for clinicians, with higher predictive accuracy according to the current understanding of type 2 diabetes, matching genetic and lifestyle data. The two new models of disease architecture were (1) the ‘palette’ model, centred on a molecular taxonomy, which contributes to diabetes risk and progression, in opposition to the ‘pigeonhole’ that is based on ‘You have type 2F diabetes; your diabetes is caused by a defect in x and the correct treatment is y’, and (2) the DIRECT-DETECT prediction model based on biomarkers for glycaemic deterioration aimed at identifying non-diabetic individuals who are at high risk of rapid, short-term

glycaemic deterioration and predict new-onset diabetes glycaemic deterioration in people who have recently been diagnosed with type 2 diabetes (185,186,211).

Joint vision of the applicability of these projects in Health

Based on the funded-projects for diabetes, considering both the results collected and their objectives, and taking into account the global population, two inter-dependent paths must be executed in parallel, the first one being more scientific-oriented and the second one medical-oriented, in order to slow the increasing prevalence, as illustrated in Figure 25.

The scientific dimension would include the acquisition of more biological samples and genetic data, in the states of the individual's health as healthy and sick, and with the help of SUMMIT, IMIDIA, EBiSC, StemBANCC, EMIF and IMI2PACT promote the research on β-cells as well as validation of new biomarkers, genetic markers, patient stratification, discover more molecular mechanisms/pathways and develop new treatments for T1D, T2D and diabetic complications. Besides, this approach aims at conducting clinical studies with more safety and efficacy endpoints available by SUMMIT, DIRECT, INNODIA and BEAT-DKD, to allow a marketing authorisation of innovative medicines/therapeutics in a shorter time, less expensive and more focused way on personalised medicine.

The medical dimension would include the use of predisposition markers developed by IMIDIA, DIRECT and INNODIA in order to identify people at higher risk of developing diabetes, with a particular interest in T2D, promoting the possibility of early intervention mainly in lifestyle habits, diet and physical exercise, and thus delaying the disease. Following the natural cycle of the disease, the objective would be to diagnose the recent-onset patients, through imaging technologies, tools and patient-centred models for clinicians developed by DIRECT, IMIDIA, INNODIA and RHAPSODY. After the identification, through the application of the stratification models of DIRECT, INNODIA and RHAPSODY/BEAT-DKD and the inter-individual factors in response variation to the therapeutic agents, identified by SUMMIT and DIRECT, characterize the subtype of patient. The treatment selection would be performed by using the patient’s stem cells to screen the medicine and dosage more effective and safer (preventing chronic toxicity

effects). The monitoring of disease progression would be possible in case of implementation of biomarkers, genetic markers and tools created by SUMMIT, IMIDIA, DIRECT, INNODIA, EMIF and RHAPSODY, with adaptations of pharmacological treatment dose or medicines changes in case of inadequate response. Additionally, with the use of biomarkers, genetic factors and tools developed by SUMMIT and BEAT-DKD, it would be possible to identify patients who during the progression of the disease, are more likely to develop diabetic complications, allowing to act in advance. With the application of imaging technologies developed by SUMMIT and EMIF and the information provided by BEAT-DKD, LITMUS, Hypo-RESOLVE and CARDIATEAM, it would also be possible to advance the identification of patients with diabetic complications, especially diabetic nephropathy, diabetic retinopathy, cardiovascular disease, hypoglycaemia and NAFLD. Through the use of genetic factors and biomarkers developed by SUMMIT and BEAT-DKD, it would be desirable employ the best choice of pharmacological treatment for the characteristics of the patient and obtain a follow-up of the progression of the disease to retard/stabilize the disease.

In conclusion, this integrated vision model supports a clinical model directed primarily and mainly at prevention, through the individual genetic and biological knowledge; as a first-line intervention, acting in the delay of diabetes onset; and in cases of diagnosis, to promote treatment according to the subtype of patients and monitor the progression of the disease. Only in this way, it will be possible to decrease the incidence and mortality rate of diabetes, provide an increase in the patient's quality of life, ensure sustainable people-centred health systems, and minimize direct and indirect diabetes-related cost in health systems.

The biggest bottleneck will be the implementation of this model in clinical practice. We believe that this will require the involvement of the entire population, including healthcare professionals, with awareness-raising and training actions, as well as the creation of pilot projects in the European countries.

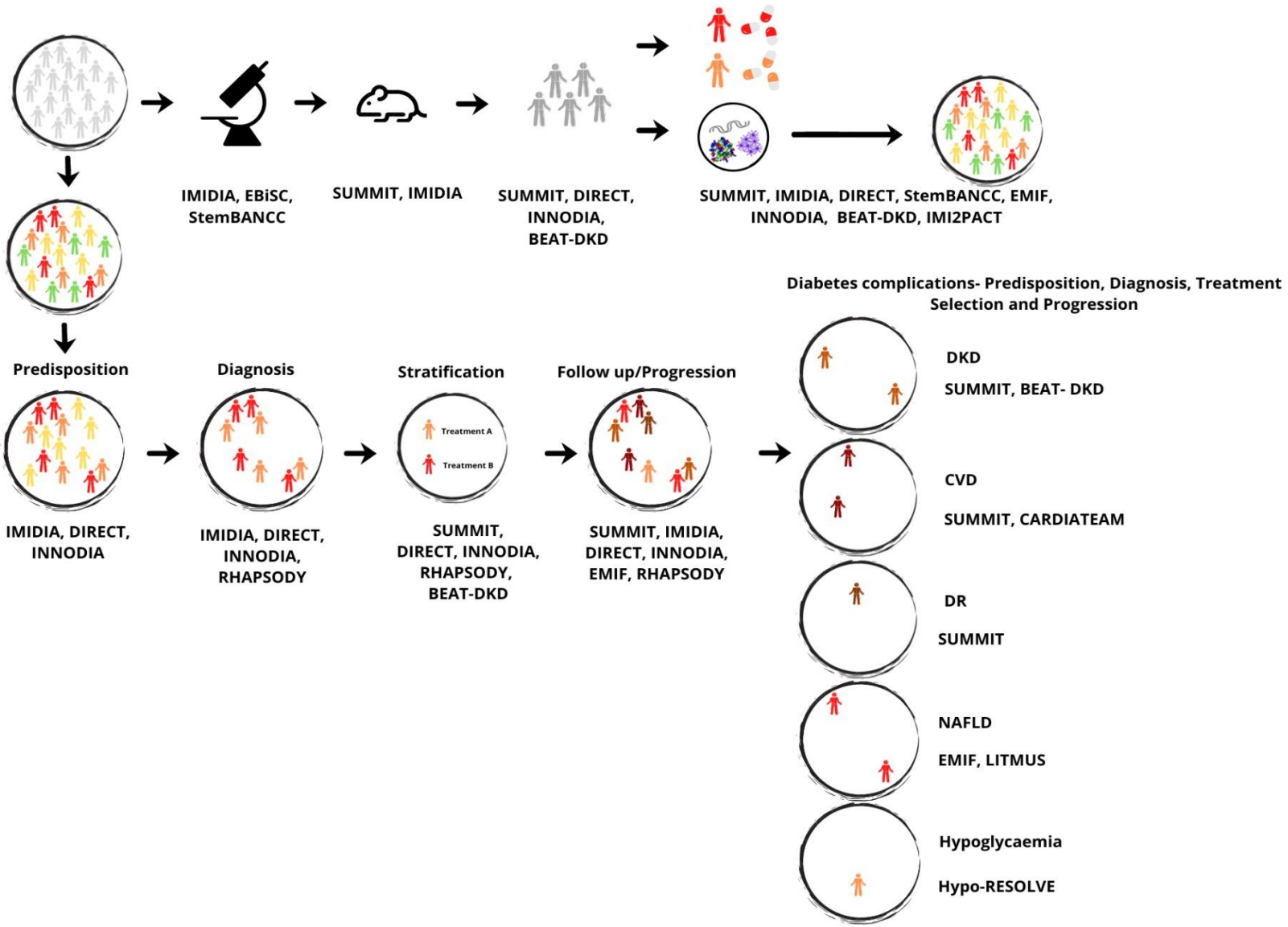

Figure 25 - Based on the funded-projects, the slowing of diabetes' prevalence will be possible through the development of two inter-dependent pathways. One is scientific-oriented and is focused on the development of new medicines, as well as biomarkers and genetic factors that allow the identification of the predisposition risk. The second is medical-oriented, aiming to enable early diagnosis, treatment selection according to patient stratification, follow-up and prevention of diabetes complications.

Weaknesses

In accordance with other projects funded by the EU's framework, those under the IMI1 and IMI2 programmes aim to publish the results obtained, thus enabling and fostering open science, create innovative products and services for others academia, industry and biotech companies, and stimulate growth across Europe.

However, when collecting data on diabetes projects, we found a large gap in their publications library as these did not include all the articles, mainly in SUMMIT, IMIDIA and DIRECT.

As illustrated in Table 3, on SUMMIT website, there were only publications between 2010 and 2014, however, on the Cordis database, we found publications up to 2018. Similarly, although the IMIDIA website only included publications from 2011 and 2012, the Cordis database had articles until 2014.

Regarding the DIRECT project, its website had only assembled the publications of the participating companies, all published before obtaining funding.

Besides, another issue identified in some project libraries was the duplication of articles.

Table 3 - Presentation of the Year of publications presented on project´s library during the literature search.

Project	Timeline	Year of publications presented in the project libraries
SUMMIT	2009-2015	2010-2014
IMIDA	2010-2015	2011-2012
DIRECT	2012-2019	2003-2011
StemBANCC	2012-2018	2013-2018
EMIF	2013-2018	2013-2018
EBiSC	2014-2017	2016-2019

INNODIA	2015-2022	2016-2019
RHAPSODY	2016-2020	2017-2019
BEAT-DKD	2016-2021	2016-2019
LITMUS	2017-2022	---
Hypo-RESOLVE	2018-2022	---
IMI2PACT	2019-2023	---
CARDIATEAM	2019-2024	---

In order to obtain a complete list of publications, we contacted the project's coordinators and co-ordinators without successful response in the vast majority of projects. The exceptions were the projects DIRECT and Hypo-RESOLVE.

Additionally, although the Cordis database was used to supplement the list of publications gathered from the project websites, this database was also not updated and complete. Since none of the data sources was complete, this was considered as the major limitation of this dissertation, due to the fact that it may not accurately reflect all the results produced by IMI-funded projects for diabetes.

Lastly, we also verified the occurrence of results replication between projects, evidencing the lack of information exchange between them.

Strengths

Concerning the strengths, it is important to highlight the importance of the IMI website as a crucial tool throughout this literature review, since it was the only source that clearly and unequivocally presented the compilation of the funded projects with an indication of their aims, participants, third parties, contribution and total costs, contacts of the project coordinator and co-coordinator, project website and crucial documents such as the final report of SUMMIT and EBiSC.

Additionally, some projects created joint ventures in order to promote information sharing, a robustness fact that should be highlighted. This was the case of "IMI Diabetes Platform", composed by IMIDIA, DIRECT and SUMMIT, which is one of the world's leading initiatives in this area, focusing on the development of new solutions to improve disease management (Figure 26) (212). Another important case includes the RHAPSODY/BEAT-DKD partnership that through their joint action have succeeded in establishing a group of patients with different levels of risk for certain complications associated with diabetes.

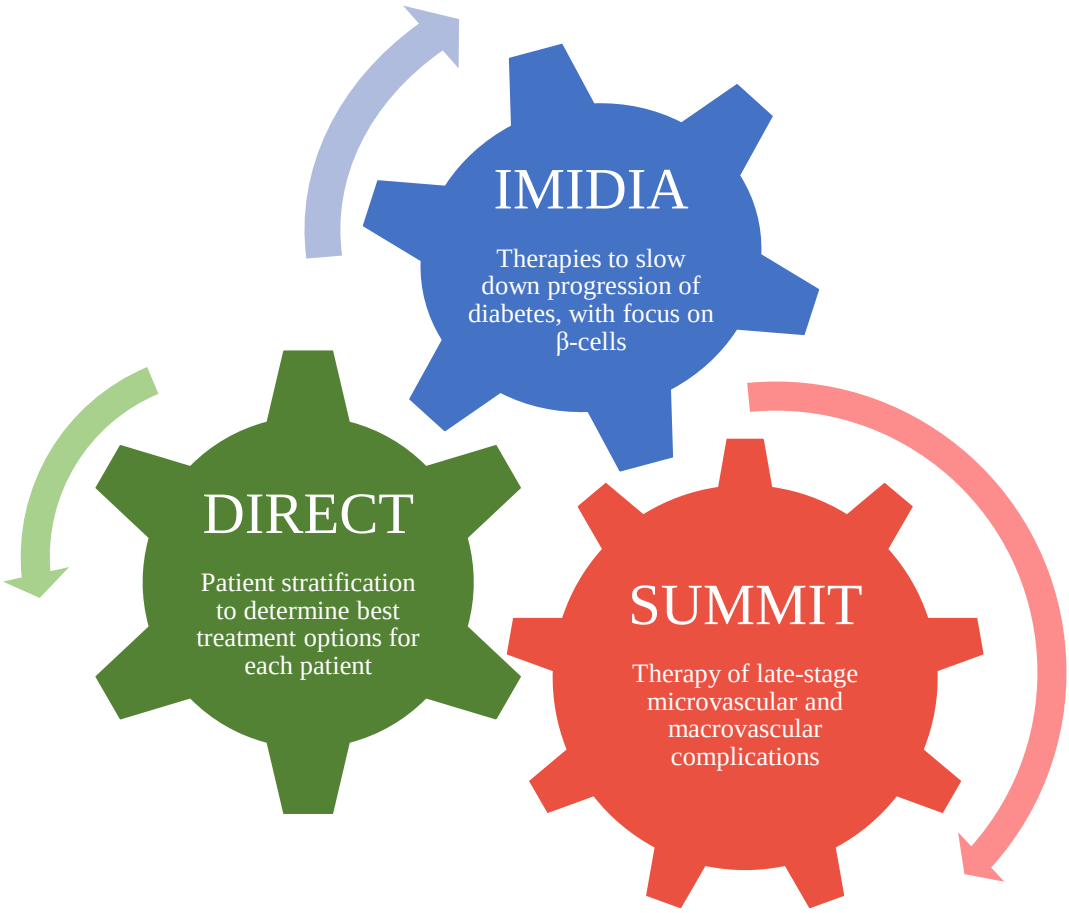

Figure 26 - Key-aspects of the projects that constitute the IMI DIABETES PLATFORM, namely SUMMIT, IMIDIA and DIRECT.

Conclusion

In order to reduce the prevalence, mortality and the economic burden of diabetes and its related complications, IMI has funded thirteen projects encompassing all dimensions of the disease, including the scientific and clinical fields. Of the thirteen projects, one was targeted to type 1 diabetes, i.e. INNODIA, three to type 2 diabetes, namely DIRECT, EMIF and RHAPSODY, four to complications of diabetes, specifically SUMMIT, BEAT-DKD, LITMUS, Hypo-RESOLVE and CARDIATEAM, and the remaining four are scientific-oriented, more precisely StemBANCC, EBiSC, IMIDIA and IMI2PACT.

The results were presented by integrating them into the recommendations defined by the SRA in respect of each of the four main axes of the IMI2 programme, which correspond to target & biomarker identification, innovative clinical trial paradigms, innovative medicines and patient-tailored adherence programmes.

It was found that the vast majority of the data concerned the "Target & Biomarker identification" axis. In this pillar, from the ten recommendations outlined, those achieved were 1) identify and validate biological markers, tools and assays, 2) determinants of inter-individual variability, 3) understand the molecular mechanisms underlying the disease, 4) develop a platform of pre-clinical assays and 5) develop systems models.

A wide range of biomarkers have been identified for type 1 diabetes (INNODIA), type 2 diabetes (SUMMIT, IMIDIA, DIRECT and EMIF), pancreatic β-cells (IMIDIA and RHAPSODY), DKD (SUMMIT and BEAT-DKD), CVD, DR and LEAD (SUMMIT).

Regarding novel tools, five categories were established according to their purpose, namely related to diabetes (IMIDIA, DIRECT and RHAPSODY), type 1 diabetes (INNODIA), type 2 diabetes (EMIF), diabetic complications (SUMMIT), and genetic research (SUMMIT and StemBANCC).

Concerning the novel determinants of inter-individual variability, SUMMIT, IMIDIA, DIRECT, EMIF, INNODIA and RHAPSODY proposed a significant number of genetic markers for diabetes and its complications, BEAT-DKD identified a non-genetic inter-individual therapeutic variability factor, DIRECT referred the influence of gut composition, age of diagnosis and BMI factors on the onset of diabetes, and, at last,

EMIF showed the association with other factors such as ethnicity and metabolic health. Moreover, two models of patient stratification were proposed, one by INNODIA for glycaemic control in patients with T1D and another by RHAPSODY and BEAT-DKD related with the identification of the patient's risk level for certain diabetic complications.

Furthermore, novel relevant pathways were proposed for β-cell development and function (IMIDIA), type 1 diabetes (INNODIA), type 2 diabetes (DIRECT and EMIF), CVD (SUMMIT), DKD (BEAT-DKD), DR (SUMMIT), endometrial cancer risk (EMIF), dementia (EMIF), NAFLD (EMIF), anorexia (INNODIA), bulimia (INNODIA) and ADHD (INNODIA).

In terms of pre-clinical studies, StemBANCC, EBiSC and IMIDIA proposed innovative iPSCs lines derived from diabetes and created their own databases; StemBANCC, INNODIA and RHAPSODY developed three catalogues, namely β-cell' Bi-DOCS, HLA-I peptidome of β-cells and cis-eQTLs for T2D; IMIDIA, StemBANCC and RHAPSODY established several protocols intended to improve the reliability of laboratory studies; and SUMMIT and IMIDIA developed new specific animal models.

Concerning systems models, two new *in silico* models were generated by SUMMIT, one for clinical complications in T1D and other for aspirin action, and BEAT-DKD proposed three models associated with DKD, namely the *Drosophila* nephrocyte, the systems biology and an *in-silico* analysis.

For the "innovative clinical trial paradigms" axis, the data applied two of the twelve recommendations defined, especially 1) utilise innovative endpoints, trial designs, simulation and analytical approaches to devise new clinical trial paradigms and 2) develop innovative clinical endpoints. In particular, two novel patient-centred design models were proposed, one by SUMMIT and a second by BEAT – DKD: the GBR and umbrella, respectively. With respect to innovative clinical endpoints, SUMMIT validated a prediction model for T2D, DIRECT-DETECT, which may be used in the selection process in clinical trials.

Regarding innovative medicines, from the eleven recommendations, those with results were: 1) identify new or alternative therapeutic concepts (targets) for treatment and prevention of disease, 2) develop novel therapeutic agents and disease prevention strategies, and 3) as implement new approaches for the development and production of biopharmaceuticals and tissue engineering. The projects that suggested novel potential

therapeutic targets were SUMMIT, IMIDIA, StemBANNC, EMIF and INNODIA. In terms of novel pharmacologic agents for T2D, SUMMIT developed the clinical trials of Aleglitazar, RAAS inhibitors, supported the use of low-dose aspirin for the secondary prevention of cerebro-cardiovascular events. In addition, DIRECT demonstrated the cardio-metabolic benefit of metformin, BEAT-DKD supported the clinical efficacy of SGLT2 inhibitors and GLP1R agonists in diabetic patients with DKD, and StemBANCC established four different stem cell-based replacement treatments.

About large-scale production of human pluripotent stem cell, StemBANCC demonstrated that the continuous peristaltic pump-based circulation technology, in a hydraulically driven bioreactor, can be a potential 3D tool and a key in this process.

At last, from the seven recommendations, the data collected for maximizing patient-tailored adherence programmes correspond to only one, namely develop patient-centred predictive models, having developed two screening tools for T2D, namely the 'palette' model and DIRECT-DETECT.

Additionally, in order to promote the implementation of the results obtained in the scientific community, we present a model that conveys an integrated approach of IMI funded projects, highlighting the importance of developing new and accurate biomarkers and genetic markers that allow the identification of the predisposition of the individual, as well as innovative and specific medicines for the clinical conditions of the type of patient. Concerning clinical practice, our model is focused on preventing the onset of diabetes and, when it is not possible and the individual develops the disease, enable the existence of early diagnosis and stratification of the patient to retard the progression of the disease and prevent the development of diabetic complications.

In terms of future perspectives, the biggest bottleneck will be the implementation of the proposed joint vision model, or a similar one, into the clinical practice, although all IMI-funded projects highlight this trend as the only one able to provide an effective response in the treatment of chronic diseases, in particular diabetes, and there are already proves of shifts in the paradigm. Nevertheless, the involvement of key stakeholders, including patients, will always be essential to the success of this process.

In conclusion, based on the analysis of the information mentioned, it is possible to conclude that the objectives proposed for the dissertation have been achieved, allowing

a clear perception of the role of IMI in the scientific advances that have occurred in recent years, focused on personalized medicine.

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Supporting Information

Appendix 1: Brief Description of IMI-funded projects in diabetes

Project	Call	Contribution	Duration	Grant number	Objectives
SUMMIT: Surrogate markers for micro- and macro-vascular hard endpoints for innovative diabetes tools (closed)	IMI 1 – Call 1	IMI Funding (14 654 559) EFPIA in kind (15 252 050) Other (4 905 472) Total Cost: 34 812 081€	01/11/2009 to 31/10/2015	115006	(1) Identify and characterize markers associated to diabetic nephropathy, diabetic retinopathy, and Lower Extremity Arterial Disease in both type 1 and type 2 diabetes as well as on cardiovascular disease in type 2 diabetes, to predict risks of developing the complications and monitor the effects of therapeutic interventions. (2) Develop knowledge, procedures, technologies and tools to make clinical trials testing of novel medications in diabetic complications shorter and more focused.
IMIDIA: Improving beta-	IMI 1 – Call 1	IMI Funding (8 060 760)	01/02/2010 to	115005	(1) Deliver novel tools for the

cell function and identification of diagnostic biomarkers for treatment monitoring in diabetes (closed)		EFPIA in kind (16 940 659) Other (2 445 590) Total Cost: 27 447 009€	30/09/2015		study of human beta-cell development, function and survival, their modulation by potential therapeutic compounds, and for *in vivo* beta-cell imaging. (2) Deliver biomarkers for the diagnosis and prognosis of beta-cell failure and for monitoring diabetes progression and treatment. (3) Deliver knowledge on novel molecular pathways and sites that control beta-cell life and death as well as mass and function.
DIRECT: Diabetes research on patient stratification (ongoing)	IMI 1 – Call 3	IMI Funding (21 388 643) EFPIA in kind (18 816 527) Other (6 278 957) Total Cost: 46 784 127€	01/02/2012 to 31/07/2019	115317	(1) Identify the subtypes of type 2 diabetes patients. (2) Identify and validate biomarkers associated with different subtypes of type 2 diabetes and different rates of disease progression. (3) Determine the most appropriate

					treatments for each subtype of type 2 diabetes patients.
StemBANCC: Stem cells for biological assays of novel drugs and predictive toxicology (closed)	IMI 1 – Call 4	IMI Funding (26 000 000) EFPIA in kind (20 761 386) Other (8 249 094) Total Cost: 55 010 480€	01/10/2012 to 31/03/2018	115439	(1) Generate high-quality human induced pluripotent stem cell lines to study a range of chronic diseases (peripheral neuropathies, neurodegenerative disorders, neurodysfunctional disorders, diabetes) and test for drug efficacy and safety. (2) Characterize iPS cell lines in terms of their genetic, protein, and metabolic profiles.
EMIF: European Medical Information Framework (closed)	IMI 1 – Call 4	IMI Funding (24 356 096) EFPIA in kind (24 354 503) Other (7 073 712) Total Cost: 55 784 311€	01/01/2013 to 30/06/2018	115372	(1) Develop a common information framework of patient-level data that will link up and facilitate access to diverse medical and research data source. (2) Identify predictors of metabolic

					complications in obesity. (3) Identify predictors of Alzheimer´s disease in the pre-clinical and prodromal phase.
EBiSC: European Bank for induced pluripotent Stem Cells (closed)	IMI 1 – Call 8	IMI Funding (21 840 380) EFPIA in kind (7 167 072) Other (5 320 406) Total Cost: 34 327 858€	01/01/2014 to 31/12/2017	115582	(1) Establish a European iPS cell bank that will be the 'go-to' resource for the characterisation, storage and distribution of high-quality iPS cells.
INNODIA: Translational approaches to disease modifying therapy of type 1 diabetes: an innovative approach towards understanding and arresting type 1 diabetes. (ongoing)	IMI 2 – Call 1	IMI Funding (17 630 000) EFPIA in kind (12 745 192) Associated Partners (9 164 968) Other (633 006) Total Cost: 40 173 160€	01/11/2015 to 31/10/2022	115797	(1) Advance the understanding of type 1 diabetes and address the lack of tools and technologies that will allow clinicians to predict, evaluate and prevent the onset and progression of type 1 diabetes. (2) Perform clinical intervention studies leading to novel therapies for preventing and curing type 1 diabetes.

RHAPSODY: Assessing risk and progression of prediabetes and type 2 diabetes to enable disease modification. (ongoing)	IMI 2 – Call 3	IMI Funding (8 130 000) EFPIA in kind (8 379 249) Other (2 189 500) Total Cost: 18 698 749€	01/04/2016 to 31/03/2020	115881	(1) Understand the factors that drive the progression of pre-diabetes to diabetes and the deterioration of the condition of people with diabetes. (2) Develop novel biomarkers to refine diagnosis leading to better patient stratification, promote prevention, and support innovative drug discovery for personalized management of type 2 diabetes.
BEAT-DKD: Biomarker enterprise to attack DKD (ongoing)	IMI 2 – Call 5	IMI Funding (15 085 937) EFPIA in kind (13 226 100) Associated Partners (1 850 999) Other (1) Total Cost: 30 163 037€	01/09/2016 to 31/08/2021	115974	(1) Provide a holistic systems medicine view of the pathogenesis DKD to identify targetable mechanisms and pathways underlying initiation and progression of DKD, applying a novel sub-classification of diabetes. (2) Identify and validate biomarkers of disease

					progression and treatment responses representing the first steps towards precision medicine in the management of DKD.
LITMUS: Liver Investigation: Testing Marker Utility in Steatohepatitis (ongoing)	IMI 2 – Call 9	IMI Funding 15 797 881 EFPIA in kind 24 180 663 Other 7 302 863 Total Cost 47 281 407€	01/11/2017 to 31/10/2022	777377	(1) Develop, robustly validate and advance towards regulatory qualification biomarkers that diagnose, risk stratify and/or monitor NAFLD and NASH progression and fibrosis stage. (2) Develop and validate imaging techniques that will allow doctors and researchers to rapidly and easily diagnose the severity of patients' disease and monitor changes in patients' livers.
Hypo-RESOLVE: Hypoglycaemia - REdefining SOLutions for better liVEs	IMI 2 – Call 10	IMI Funding (13 450 057) EFPIA in kind (10 316 000) Associated Partners	01/05/2018 to 30/04/2022	777460	(1) Provide researchers and clinicians with more validated data about the condition by creating a sustainable

(ongoing)		(3 008 525) Total Cost: 26 774 583€			clinical database, by conducting studies to better understand the underlying mechanisms of hypoglycaemia, by conducting a series of statistical analyses to define predictors and consequences of hypoglycaemia, and by calculating the financial cost in European countries.
IM2PACT: Investigating mechanisms and models predictive of accessibility of therapeutics into the brain	IMI 2 – Call 12	IMI Funding (9 000 000) EFPIA in kind (8 410 136) Total Cost: 17 410 136€	01/01/2019 to 31/12/2023	807015	(1) Advance the understanding of the blood-brain barrier to facilitate the development of more effective treatments for a range of neurological and metabolic disorders; (2) Develop better models of the BBB so that researchers can study it more easily; (3) Investigate the biology of the BBB in both health

					and disease, and the transport routes across it; (4) Develops innovative systems capable of delivering medicines to the brain.
CARDIATEAM: Cardiomyopathy in type 2 diabetes mellitus (ongoing)	IMI2 – Call 13	IMI Funding (6 700 000) EFPIA in kind (6 000 000) Other (182 500) Total Cost: 12 882 500€	01/03/2019 to 29/02/2024	821508	(1) Determine how type 2 diabetes represents a central mechanism contributing to the pathogenesis and progression of diabetic cardiomyopathy. (2) Determine how distinct diabetic cardiomyopathy is from other forms of heart failure.

Appendix 2: List of DIRECT publications

Nature of Communication	Title	Responsible Participant	Date	Target audience
Publications				
Peer Review Journal Article: Ahmad S et al. PLoS Genet. 2013;9(7):e1003607. doi: 10.1371/journal.pgen.1003607. Epub 2013 Jul 25. PubMed PMID: 23935507	Gene × physical activity interactions in obesity: combined analysis of 111,421 individuals of European ancestry	15/ULUND	25 Jul 2013	Diabetes researchers and practitioners
Peer Review Journal Article: Koivula et al. Diabetologia. *Provisionally accepted*	Discovery of biomarkers for glycaemic deterioration before and after the onset of type 2 diabetes: rationale and design of the epidemiological studies within the IMI DIRECT consortium	15/ULUND	Submitted Dec 2013	Diabetes researchers and practitioners
Peer Review Journal Article: Zhou et al. Diabetes Care. 2013 Nov 1. [Epub ahead of print] PubMed PMID: 24186880	Clinical and genetic determinants of progression of type 2 diabetes: A DIRECT Study	02/UNIVDUN	01 Nov 2013	Diabetes researchers and practitioners
Peer Review Journal Article: Franks et al. Diabetes Care. 2013 May;36(5):1413-21. doi: 10.2337/dc12-2211. Review. PubMed PMID: 23613601	Gene-environment and gene-treatment interactions in type 2 diabetes: progress, pitfalls, and prospects	15/ULUND, 02/UNIVDUN	May 2013	Diabetes researchers and practitioners
Rouskas et al. SOARD. Provisionally accepted	Weight loss independent association of TCF7L2 gene polymorphism with fasting blood glucose after Roux-en-Y Gastric Bypass in type 2 diabetes patients	26/CHRU LILLE	Submitted Dec 2013	Diabetes researchers and practitioners
Pasquali et al. Nature Genetics 2014 Jan 12	Pancreatic islet enhancer clusters enriched in Type 2 diabetes risk-associated variants.	08/IDIBAPS, 19/UOXF	Jan 2014	Scientists

[Epub ahead of print]. PMID: 24413736.				
Peer Review Journal Article: Koivula et al. Diabetologia (PMID: 24695864)	Discovery of biomarkers for glycaemic deterioration before and after the onset of type 2 diabetes: rationale and design of the epidemiological studies within the IMI DIRECT consortium	ULUND	Published 2014 Apr	Diabetes researchers and practitioners
Manuscript: Journal for Diabetes Health Professionals in The Netherlands (in Dutch)	Will DNA be of help in treating type 2 diabetes patients?	LUMC	May	Health professionals
Journal article	Poveda A, Koivula RW, Ahmad S, Barroso I, Hallmans G, Johansson I, Renström F, Franks PW. Innate biology versus lifestyle behaviour in the aetiology of obesity and type 2 diabetes: the GLACIER Study. Diabetologia. 2015 Dec 1. [Epub ahead of print] PubMed PMID: 26625858.	ULUND	01 Dec 2015	Scientific / Clinical Community
GBR paper	A paper on the methodological features of genotype-based recall trial, which underpins potential clinical trial designs for Stage 2 of DIRECT (in review, Diabetes Care)	ULUND, UNIVDUN	10 Dec 2015	Scientific / Clinical Community
GBR website	A web tool to help design special randomized controlled trials for testing precision medicine hypotheses will be published in the above paper and will be of immediate value to DIRECT when designing the Stage 2 trials	ULUND, UNIVDUN	24 April 2015	Scientific / Clinical Community
Journal article	Statistical power considerations in genotype-based recall randomized controlled trials.	Atabaki-Pasdar N, Ohlsson M, Shungin D, Kurbasic A, Ingelsson E, Pearson ER, Ali A, Franks PW.	Nature Sci Rep. 2016 Nov 25;6:37307. doi: 10.1038/srep37307. PMID: 27886175	Scientific community
Journal article	Causal inference in obesity research.	Franks PW, Atabaki-Pasdar N.	J Intern Med. 2016 Dec 8. doi: 10.1111/joim.12577. [Epub	Scientific community

			ahead of print] Review. PMID: 27933671	
Journal article	Lifestyle and precision diabetes medicine: will genomics help optimise the prediction, prevention and treatment of type 2 diabetes through lifestyle therapy?	Franks PW & Poveda A.	Diabetologia Epub Jan 2017	Scientific community
Journal article	Exposing the exposures in type 2 diabetes.	Franks PW & McCarthy MI	Science. 354(6308): 69-73. 2016	Scientific community
Journal article	Putting the genome in context: gene-lifestyle interactions in type 2 diabetes.	Franks PW & Pare G.	Curr Diab Rep. 6(7):57. 2016	Scientific community
Journal article	Sustained influence of metformin therapy on circulating glucagon-like peptide-1 levels in individuals with and without type 2 diabetes.	Preiss D, Dawed A, Welsh P, Heggie A, Jones AG, Dekker J, Koivula R, Hansen TH, Stewart C, Holman RR, Franks PW, Walker M, Pearson ER, Sattar N DIRECT consortium group	Diabetes Obes Metab. 2017 Mar;19(3): 356-363. doi: 10.1111/dom.12826. PMID: 27862873.	Scientific community
Journal article	Predicting glycated hemoglobin levels in the non-diabetic general population: Development and validation of the DIRECT-DETECT prediction model - a DIRECT study.	Rauh SP, Heymans MW, Koopman AD, Nijpels G, Stehouwer CD, Thorand B, Rathmann W, Meisinger C, Peters A, de Las Heras Gala T, Glümer C, Pedersen O, Cederberg H, Kuusisto J, Laakso M, Pearson ER, Franks PW, Rutters F, Dekker JM.	PLoS One. 2017 Feb 10;12(2):e0171816. doi: 10.1371/journal.pone.0171816. PMID: 28187151	Scientific community

Journal article	Personalized medicine in diabetes: the role of 'omics' and biomarkers.	Pearson ER. /UNIVDUN	Diabet Med. 2016 Jun;33(6):712-7. doi: 10.1111/dme.13075. Review.PMID: 26802434	Scientific community
Journal article	Painting a new picture of personalised medicine for diabetes	Mark McCarthy / UOXF	Diabetologia 2017 Feb 7 doi: 10.1007/s00125-017-4210-x. [Epub ahead of print]	Scientific community
Journal article	A Genome-Wide Association Study of IVGTT-Based Measures of First-Phase Insulin Secretion Refines the Underlying Physiology of Type 2 Diabetes Variants	Wood AR, Diabetes Research on Patient Stratification (DIRECT), et al	Diabetes. 2017 Aug;66(8):2296-2309. doi: 10.2337/db16-1452.	Scientific community
Journal article	Integrative network analysis highlights biological processes underlying GLP-1 stimulated insulin secretion: A DIRECT study	Gudmundsdottir V, Pedersen HK, Allebrandt KV, Brorsson C, van Leeuwen N, Banasik K, Mahajan A, Groves CJ, van de Bunt M, Dawed AY, Fritsche A, Staiger H, Simonis-Bik AMC, Deelen J, Kramer MHH, Dietrich A, Hübschle T, Willemsen G, Häring HU, de Geus EJC, Boomsma DI, Eekhoff EMW, Ferrer J, McCarthy MI, Pearson ER, Gupta R, Brunak,	PLoS One. 2018 Jan 2;13(1):e0189886. doi: 10.1371/journal.pone.0189886. PubMed PMID: 29293525.	Scientific community

		't Hart LM.		
Journal article	Metabolite ratios as potential biomarkers for type 2 diabetes: a DIRECT study	Molnos S, Wahl S, Haid M et al.	Diabetologia. 2018 Jan;61(1):117-129. doi: 10.1007/s00125-017-4436-7	Scientific community
Journal article	Long-Term Stability of Human Plasma Metabolites during Storage at −80 °C	Haid M et al.	J. Proteome Res., 2018, 17 (1), pp 203–211	Scientific community
Journal article	Mass spectrometry based qualification of antibodies for plasma proteomics	Claudia Fredolini, Sanna Bystrom et al.	https://www.biorxiv.org https://doi.org/10.1101/158022	Scientific community

Appendix 3: Summary table of the data collected from the IMI-funded projects for Diabetes

Target & Biomarker Identification				
1. Identify and validate biological markers, tools, and assays				
Novel Biomarkers				
	DKD	**CVD**	**DR and LEAD**	**T2D**
SUMMIT	Free and total desmosines exhibited utility for DKD disease progression, patient stratification and medicine efficacy (62). Fourteen biomarkers that improve the prediction of rapid progression of estimated glomerular filtration rate (eGFR) decline in T2D (65). It was suggested an inverse relationship between serum UMOD and risk of DKD (65).	A panel of six biomarkers was associated with CVD: Apolipoprotein CIII, Interleukin-6, Interleukin-15, high sensitivity Troponin T, N-terminal prohormone B-type natriuretic peptide and soluble receptor for advanced glycation end products (65). N-terminal prohormone B-type natriuretic peptide and Apolipoprotein CIII improved prediction of CVD (65). MMP-7 and MMP-12 were associated with vascular complications in T2D (65). Interleukin-5 was proposed as a potential plasma biomarker for early subclinical atherosclerosis in women due to the significant inverse association with changes in intima-media thickness (213). Proof of lack of evidence between the role of vitamin D levels in early subclinical	VEGFA, NFAT, caspase-3 activation and advanced glycation end products were associated with the development of diabetic retinopathy (63–65). Caspase-3 activation is generally seen in animal models with either inherited or induced photoreceptor degeneration (63).	No evidence for the association of lower adiponectin levels and type 2 diabetes risk (61).

		atherosclerosis (214).		
IMIDIA	**T2D**			
	High free fatty acids levels were associated with the risk of T2D (48).			
DIRECT	**T2D**			
	Valine to phosphatidylcholine acyl-alkyl C32:2 metabolite was proposed for identification of individuals at high-risk (49).			
EMIF	**T2D**			
	Several biological markers were suggested to predict the risk of type 2 diabetes, more precisely gamma-glutamyl transpeptidase (GGT), with emphasis on small-GGT, plasma mannose and plasma metabolites, SIRT1, branched-chain amino acids, 3-Hydroxyisobutyric acid, tyrosine, alanine transaminase , aspartate aminotransferase, C-reactive protein and adiponectin. The markers suggested for white adipose tissue (WAT) inflammation were CCL18 serum levels. A full list of biomarkers identified for T2D risk by using Mendelian randomization was published in 2016 (50–59). Visceral adipose tissue and subcutaneous adipose tissue adipocytes were suggested as histological markers for T2D abnormalities (60).			
INNODIA	**T1D**			
	The novel β-cell biomarkers were DPP6, FXYD2γa, PDL1, GAD, IA-2 and ZnT8 antibodies (37–40). The novel autoantigens discovered were citrullination ER chaperone GRP78 (citGRP78), CHGA, PTPRN, GAD2, SLC30A8, IAPP and MTIF3 in patients with type 1 diabetes, and PPIL2, and MLH1 in recent-onset type 1 diabetes (41–43). Some biological factors were associated with a pro-survival role in human β-cells, among which HLA-DQ6, complement system C3, Bcl2-A1 protein, miR-375, miR-148α-3p, mir-29α-3p, ER-resident proteins THBS1, STAT3, ERK ½, and MANF protein (37,44–47).			
RHAPSODY	**β-cells**		**Hyperglycaemia**	
	The pancreatic β-cells biomarkers proposed were transcription factor MondoA, MAF and PAX6 (75,76). The β-cell dedifferentiation markers discovered were fibroblast growth factor 1, fibroblast growth factor 2, SOX9, NEUROG3, and gastrin (76).		The hyperglycaemia biomarkers suggested were SLC2A2, FFAR4, TMEM37, TMEM27, and ALDOB (77).	
BEAT - DKD	**DKD**			
	Disease Prognosis: It was identified a set of 33 compounds with the potential of reversing the expression signature of renal age-associated DKD prognosis genes. (71) Urinary epidermal growth factor was identified as an early prognostic marker for DKD rapid progression (70). **Disease initiation:** Extracellular vesicles, i.e. urinary extracellular vesicles, based protein miRNA biomarkers, quantitative renal magnetic resonance imaging T1- and T2 mapping, downregulation of p57Kip2, and KIM1, FGF23, NTproBNP, HGF, and MMP1 were validated as predictors of eGFR decline levels in patients with type 2 diabetes (66–69). **Treatment selection:** NT-proBNP can help to identify individuals who may not respond to dual RAAS inhibition with aliskiren and who may benefit from diuretic treatment or dietary sodium-lowering (72). **Neuroprotective markers:** HMOX1, NFE2L2 transcription factor, SIRT1, HSPA4, HSPA5, HSPA1A, HSPA1B, HSPB1, HSPA2, HSPB2, RARA, CCND1, TH, TSPO, TBXA2R, CXCL12, ETS1, CAT, SOD1, LCN2, IL7, IL9, IL10, IL11, VEGFA, HGF, IGF1, and receptor IGFR1, BDKRB1, KNG1, ADM, ADCYAP1, CALCA,			

<table>
<tr><td></td><td colspan="2">CCL2, TNFRSF1A, TNFRSF1B, MCP-1, and DCXR. This set is available at NCBI's PubMed (73,74).</td></tr>
<tr><td colspan="3">Novel Tools</td></tr>
<tr><td rowspan="2">SUMMIT</td><td>Diabetic complications</td><td>Genetic research</td></tr>
<tr><td>Two diagnostic tools for CVD were developed, more precisely Ultrasound Plaque Structure Analysis (UPSA)-system, a non-invasive, low cost and safe method that enables the assessment of atherosclerosis plaque structure, the detection of patients at high risk of a heart attack or stroke, and the monitoring individual's response to interventions (91) and 18F-fluoromethylcholine (18F-FMCH), a potential tracer for the evaluation of atherosclerotic vascular inflammation in diabetes (92).
For DR, it was developed one diagnostic tool, the Optical Coherence Tomography (OCT), a large dataset composed by macular thickness measurements to improve prediction of diabetic retinopathy (65).</td><td>For identification of SNPs selection and multivariate analysis, it was defined and validated four new tools, specifically Hierarchical Naïve Bayes (HNB), Binary Outcome Stochastic Search (BOSS), 'Bag of Naïve Bays' (BoNB) and Algorithm based on a Bivariate Cumulative Statistic (ABACUS) (93–97).</td></tr>
<tr><td>IMIDIA</td><td colspan="2">Diabetes
It was established a glucose nanosensor for intracellular glucose imaging, FLII12Pglu-700μ-δ6, a crucial tool for real-time analysis of glucose-induced insulin secretion in pancreatic beta cells and understand defects in diabetic conditions (78–83).
It was developed a manganese-enhanced high field magnetic resonance imaging tool suitable for diagnostic and monitoring diabetes progression (78–83).</td></tr>
<tr><td>DIRECT</td><td colspan="2">Diabetes
GADA test was proposed for prediction of diabetes progression due to the findings that individuals with GADA-positive progress approximately two times faster than other individuals with this disease (84).</td></tr>
<tr><td>StemBANCC</td><td colspan="2">Genetic research
T2D phenotypic-linkage network (T2D-PLN) was developed to integrate functional genomics data type and provide an accurate prediction of gene influence on T2D risk (98).
It was verified an upgraded of 'Genome editing' technologies used on genetic research to generate isogenic control lines, highlighting the CRISPR-Cas9. (99)</td></tr>
<tr><td>EMIF</td><td colspan="2">T2D
"Lipid triplet test" that predicts the amount of liver fat and was proposed to follow-up of T2D patients with insulin treatment to control hepatic glucose production once this is directly proportional to liver fat, independent of obesity and other factors (89,90).</td></tr>
</table>

<table>
<tr><td rowspan="2">INNODIA</td><td colspan="3">T1D</td></tr>
<tr><td colspan="3">There were developed three tools, a Dried Blood Spots method to measure C-peptide in children and adolescents recently diagnosed and to monitor β-cell function over the time at T1D risk individuals (87), a novel algorithm for the β-cell function to predict the pathway of β-cell function in recent-onset T1D patients and it would inform which intervention is more beneficial for each patient (88), and a paramagnetic contrast agent, Gd-DOTA-P88, that is based on a peptide (P88) that targets FXYD2γa, a β-cell biomarker, enabling its screening (38).</td></tr>
<tr><td rowspan="2">RHAPSODY</td><td colspan="3">Diabetes</td></tr>
<tr><td colspan="3">It was developed a machine learning developed by RHAPSODY to predict the glucose tolerance status by 22 baseline variables obtained at fasting blood acquisition (85).</td></tr>
<tr><td colspan="4">2. Determinants of inter-individual variability</td></tr>
<tr><td colspan="4">Genotype factors</td></tr>
<tr><td colspan="4">Genetic Markers</td></tr>
<tr><td rowspan="2">SUMMIT</td><td>Diabetes Mellitus</td><td>DKD</td><td>CVD</td></tr>
<tr><td>It was proposed four potential associations between resistance or diabetes and SNPs in three metabolites, more precisely glycine (rs715 at CPS1), serine (rs478093 at PHGDH), and betaine (rs499368 at SLC6A12, and rs17823642 at BHMT), and one association signal with glycine-to-serine ratio (rs1107366 at ALDH1L1) (100).</td><td>Twenty-one SNPs were associated with risk of DKD, through specific mechanisms including microalbuminuria and impairment/decrease of estimated glomerular filtration rate, such as rs7577 in CNDP2 gene, rs2346061 in CNDP1 promoter, C allele in rs1866813 at 3q22 region, rs7583877 in AFF3 gene, rs12437854 at 15q26 region, rs7588550 in the ERBB4 gene, rs10811661 in CDKN2A/B locus, rs2097443 between the PARVG and LDOC1L, rs12137135 between WNT4 and ZBTB40 genes, rs17709344 on the RGMA/MCTP2 region, rs 1670754 downstream of MAPRE1P2 gene, rs12917114 between the SEMA6D and SLC24A5, rs2838302 in the intron of SIK1 gene, ORAI isoforms downregulated, and seven SNPs across COQ5, COX6A1, GATC, TOP1MT, and PARCRG (95,120–126).
Uromodulin (UMOD) variant was associated with DKD extension (65).
It was suggested that rs4972593 is a sex-specific genetic variant that underlies women protection against end-stage renal disease in type 1 diabetes (64).
Exposed potential false-positive genetic markers previously associated with DKD (127).</td><td>Three known coronary artery calcification (CAD) SNPs, rs4977574 at 9p21, rs3825807 at ADAMTS7, and rs12526453 at PHACTR1, were associated with its development in patients with T2D (96).</td></tr>
</table>

IMIDIA	**T2D**		
	The genetic marker associated with T2D predisposition, development and progression were the rare mutation p.G1117E in the PASK gene in a single MODY family, the Q121 variant of ENPP1, intronic SNPs in TCF7L2, SNPs in ANKRD23/39, ASCL2, HHATL, NSG1, PCDH20, SCTR, CD44, FAM102B and FBXO32 gene, downregulation of ARG2, CAPN13, CHL1, FFAR4, G6PC2, PPP1R1A, SLC2A2, TMEM37, UNC5D and genes of the UPS system including UCHL1, and upregulation of KCNH8 (80,83,111–114).		
DIRECT	**T2D**		
	It was discovered that (1) allelic variation in pancreatic islet distant enhancers, i.e. cis-regulatory determinants, were specifically associated with T2D and fasting glycemia, (2) nonsense p.Arg684Ter allele in the TBC1D4 gene increased T2D risk, (3) children carrying the T2D-risk allele at some loci, such as MTNR1B and GCK, have higher birth weights and those carrying loci at ADCY5 and CDKAL1 have lower birth weights, and (4) 21 alleles associated with first-phase insulin secretion were also associated with higher type 2 diabetes risk and lower insulin secretion during intravenous glucose tolerance test, however only those in or near the MTNR1B and CDKAL1 genes showed genome-wide significance. Besides, a set of 65 BMI-associated loci was linked with type 2 diabetes. A GRS comprised of twelve obesity-predisposing gene variants, from which SNP rs1121980 in FTO locus and rs10913469 in gene yielded statistically significant individual SNP * physical activity interaction effects (115–119). Genetic variants in the TCF7L2, GLP1R, WFS1 and CTRB1/2 loci were nominally associated with GLP-1 stimulated insulin secretion (128). It was evidenced that genetic factors associated with T2D predisposition are different from those that cause their progression. Genetic variants associated with type 2 diabetes are associated with the younger onset of diabetes and younger age at insulin treatment but are not associated with diabetes progression. Moreover, GRS did not affect the period between diagnosis of diabetes and insulin requirement (84).		
EMIF	**Diabetes**		
	The increase of diabetes mellitus´ risk was associated with PCYT1A mutations, NSMCE2 mutations, DNA CpG methylation in SAT and VAT, loss-of-function mutations of PPM1K, and PIK3R1 mutations (50,59,101–104). Loss of function of EMISCD1 or ELOVL6 resulted in the expected changes in tissue fatty acid composition by increasing of C18 and Δ9 desaturated lipids associated with improved insulin sensitivity (129).		
INNODIA	**T1D**		
	The genes markers associated with the risk/development of T1D were HLA-DR3-DQ2 haplotype, HLA-DR4-DQ8 haplotype, HLA-DRB1-DQA1-DQB1 haplotype, dysregulation of miR-25-3p, miR-146α-5p, miR-21-5p, miR-21-3p, miR-93-5p, miR-23α-3p, miR-34α-5p and miR-27α-3p, DEXI, and misfolded proinsulin (44,45,107–110).		
RHAPSODY	**Diabetes**	**T2D**	**β-cells**
	Data suggested that loss-of-function mutations in TRMT10A were a monogenic cause of early-onset diabetes during adolescence or young adulthood (105). Some genes reflect the HbA_{1c} levels only at baseline and the level of glycaemic control in diabetic patients was strongly associated with immune- and cell cycle-related alterations in gene expression. A gene inversely associated with	The genetic markers associated with increased susceptibility to type 2 diabetes were SNPs near CDC123 and CDKN2Agenes, SNPs in CDKAL1, SNPs in TRIT1, SNPs in mt-tRNA and FAIM2 gene upregulated (77,106).	The discovered genetic markers associated with β-cell dedifferentiation were TNFRSF11B gene, SOX9 gene and the NEUROG3 gene (76).

	HbA_{1c} levels was PAQR7 (106).		
Other Genetic Factors			
SUMMIT	The glycaemic response to metformin varied in 34% according to the hereditable phenotype for the absolute reduction in HbA_{1c} (130). The variable rate of turn-over of the medicine target represented the main mechanism of inter-individual variability for drug-response in aspirin-treated patients with diabetes (133,134).		
DIRECT	**Regarding sulfonylureas:** Patients with HNF1A mutations that cause a form of maturity-onset diabetes to respond better to sulfonylureas than metformin, and cytochrome P450 2C9 (CYP2C9) *2 and *3 variation change sulphonylurea efficacy (20,131). **Regarding metformin:** Polymorphisms in organic cation transporter 1 alter tolerance to this medicine, (2) gene variation in SLC47A1 and ATM gene, and rs11212617 in NPAT gene modify metformin response (20,131). In obese patients with T2D, the rs7903146 in TCF7L2 gene had a significant association with a lower reduction of fasting blood glucose after Roux-en-Y Gastric Bypass surgery (132).		
Non-genetic therapeutic factor			
BEAT-DKD	Poor responders to renin angiotensin aldosterone system inhibition initiation in patients with type 2 diabetes, treated in primary care, had the highest risk of cardiovascular events and showed large variability in urinary albumin creatinine ratio. In cases with higher NT-proBNP levels aliskiren increased the risk of the cardio-renal endpoint and at lower NT-proBNP tertile, treatment with aliskiren tended to reduce cardio-renal risk (72,135,136).		
Gut composition Factors			
DIRECT	Microbiome alterations induced by metformin may promote intolerance and affect efficacy (20,131).		
Ethnicity Factors			
EMIF	South Asians have a greater prevalence of type 2 diabetes than do the background populations of the countries to which they moved, and the neonates seem to be more insulin resistant than white Europeans throughout childhood. In addition, this population's faster progression of retinopathy and nephropathy suggests they might benefit from the early use of pharmacologic treatments (137).		
Other individual factors			
DIRECT	**Age:** The individuals diagnosed younger than 50 years of age progress rapidly compared with individuals diagnosed over the age of 70. Besides, HbA_{1c} at diagnosis is higher in the younger than in the older group (84). **Sex:** Women were independently associated with rapid progression to the requirement of insulin treatment (138). **Weight:** Individuals with normal weight at diagnosis are likely to be βcell-deficient and progress rapidly to insulin requirement, and those who are particularly adipose are likely to be markedly insulin resistant (138). Moreover, higher BMI (25-30 kg/m2) and lower HDL-cholesterol (<1 mmol/l) were associated with a higher glycaemic deterioration (84). Individuals classified as metabolically unhealthy have a higher relative risk of type 2 diabetes compared with individuals classified as healthy independent of BMI category (139). **Year:** Individuals diagnosed before 2001 have a rate of deterioration higher than individuals diagnosed in or after 2006 (84). Maternal obesity during pregnancy raises the offspring's risk of type 2 diabetes (140).		
Models of patient stratification			
INNODIA	It was established five groups with distinct trajectories of glycaemic control (HbA1c) in children from age 8 and young adults with T1D. Groups differed by self-care with a lower frequency of self-monitoring of blood glucose (SMBG) in subjects with HbA1c deterioration and higher insulin dose among patients with HbA1c increase (141).		

RHAPSODY / BEAT-DKD	Identification of five subtypes of patients with different risk levels for certain complications associated with diabetes. Cluster 1 was characterised by early-onset disease, relatively low BMI, poor metabolic control, insulin deficiency, presence of GADA and was labelled as severe autoimmune diabetes (SAID); Cluster 2 was categorised as severe insulin-deficient diabetes (SIDD), was GADA negative, low age at onset, relatively low BMI, low insulin secretion and poor metabolic control; Cluster 3 was characterised as severe insulin-resistant diabetes (SIRD) was characterised by insulin resistance and high BMI; Cluster 4 was labelled by obesity but not by insulin resistance and was labelled as mild obesity-related diabetes (MOD); and finally, Cluster 5 was categorised as mild age-related diabetes (MARD), older patients and similar to cluster 4, only with modest metabolic derangements. Patients in group 2 ('severe insulin-deficient diabetes') are at the greatest risk of eye disease, while patients in group 3 ('severe insulin-resistant diabetes') had the highest incidence of kidney damage (142).	
3. Understand the molecular mechanisms underlying disease		
	CVD	**DR**
SUMMIT	It was proposed that diabetic metabolism itself did not affect the plaque development, suggesting that the lesion progression was mainly dependent on increased cholesterol levels (163). The suggestion that CVD events might occur at a lower atheroma burden in diabetes (65).	Some pathogenic factors suggested playing a role in the development of diabetic retinopathy, more specifically the vascular endothelial growth factor, which induces neovascular changes in photoreceptors, and the Nuclear Factor of Activated T-cells (63). Advanced glycation end products were also associated with the development of DR through induction of retinal expression of IL-6 which subsequently induce the expression of VEGF, the primary mediator of retinal neovascularization (63–65). Caspase-3 activation is generally seen in animal models with either inherited or induced photoreceptor degeneration (63–65). A novel phenotype form of retinopathy was identified which presented alterations in retinal morphology, such as biochemical alterations and apoptosis of neuronal and vascular cells in the retina (63).
	β-cell protection	**β-cell apoptosis**
IMIDIA	Monounsaturated fatty acids exhibited opposite effects and counteracted the toxic effects of glucolipotoxicity, induced by saturated non-esterified fatty acids and polyunsaturated fatty acids (PUFAs), more specifically docosahexaenoic acid showed to modulate lipotoxicity possibly by enhancing insulin signalling in peripheral tissues (145). ELOVL2 is a very-long-chain fatty acid elongase 2 that produces DHA and its overexpression in β-cells partially protected them from glucolipotoxicity-induced apoptosis. (145) Connexin 36 and the overexpression of H_2O_2-	Pro-inflammatory cytokines cause apoptotic beta-cell death through activation of caspase-9 and an ER specific caspase-12 that induce nitro-oxidative stress-mediated hydroxyl radical formation in the mitochondria, DNA damage and elevate cardiolipin peroxidation in β-cells (143,144). ELOVL2 downregulation significantly increased β-cell apoptosis induced by glucolipotoxicity (145).

	inactivating catalase were suggested for β-cell protection against the proapoptotic/ induced toxic effects (146,147).			
DIRECT	**T2D**			
	The progression of T2D is mediated by genetic factors, glucolipotoxicity, endoplasmic reticulum, and oxidative stress (84).			
StemBANCC	**T2D**			
	It was proposed novel pathways for pancreatic and β-cells development, during embryogenesis in rats and human, and regarding the dedifferentiation and redifferentiation process of human β-cells with the explanation of the role of key factors such as ROS, NEUROG3, MAPK1, STAT3, HIF1-α, DYRK1A, PDX1, NKX6-1, GP2, CDH1, EPCAM, SUSD2, CD142, CHGA, NEUROD, INS, and MAFA (148–153).			
EMIF	**T2D**	**Dementia**	**Endometrial cancer**	**NASH**
	Patients with NAFLD associated with TM6SF2 variant which encodes Glu167Lys (E167K) metabolically silent were suggested to had a decrease risk to develop T2D (161). The family history of T2D with one or more first-degree relatives has a 30–70% increased risk of developing the disease, however large GWAS can only explain ~10% of the risk. Data suggested that heritability of diabetes was associated with a reduced ability to store excess fat in the subcutaneous adipose tissue which, in turn, promotes ectopic fat storage (103). White adipose tissue hypertrophy was associated with insulin resistance and increased risk of developing T2D, displaying fat cell metabolic disturbances such as increased fat cell size, spontaneous lipolytic activity, and secretion of inflammatory mediators (215,216). Highlighted the role of obesity as a major risk factor for T2D, by several epidemiological (BMI), genetic (FTO gene), mechanistic (ectopic fat in key glucose metabolism organs), clinical (BMI and waist size) and	Data supported that diabetes was a risk factor of dementia by two potential pathways: dietary methyl-glyoxyl levels and/or downstream of SIRT1 (54).	T2D was associated with endometrial cancer risk because insulin decreases the levels of sex hormone-binding globulin by inhibiting its production in the liver, consequently, induces endometrial cell proliferation and decrease apoptosis, resulting in increased endometrial cancer risk. Moreover, hyperinsulinemia leads to elevated levels of free insulin-like growth factor 1 (IGF-1), which have been shown that stimulate endometrial cell proliferation (169).	T2D is one of the risk factors to develop NASH, a type of NAFLD. Patients with T2D have a 2-fold higher risk of NAFLD/NASH. (170) A fraction of 29% of the patients with NAFLD and diabetes develop NASH. (89) Genetic forms of NAFLD that predict NASH and cirrhosis were suggested, i.e. I148M variant in the PNPLA3 gene and the E167K variant in TM6SF2 gene (56). Regarding NASH, the more relevant genetic and soluble markers associated with pathology were rs738409 in PNPLA3 gene, CK-18 fragments,

	reversibility (weight loss via dietary or surgical methods) evidence (217). It was proved the presence of an early cephalic phase of insulin release in humans and neither individuals with a family history of type 2 diabetes or with impaired fasting (IFG)/ impaired glucose tolerance (IGT) exhibited a reduced early response to neural activation (218).			big-GGT (b-GGT) and s-GGT (51,89,90).
INNODIA	**T1D**	**CVD**	**Anorexia and bulimia**	**ADHA**
	The systemic autoimmune component in the disease process was supported due to the observation of the augment of autoimmunity proteins. The novel mechanisms proposed for β-cells self-destruction were through (i) secretion of ER chaperone GRP78 and its translocation to the cell surface when cytokine-exposed, (ii) IFNα which induced the overexpression of HLA class I, and (iii) dysregulation of miRNAs (42,45,154,155). DPP-4 directly affected β-cell pathophysiology (156). After the diagnosis of T1D, the β-cell specific CD^{57+}effector memory compartment of $CD8^{+}$ T-cell pool presented in the circulation calibrates to changes in β-cell function (157). A negative correlation between telomere length dynamics and glycaemic control in T1D was observed, therefore higher HbA_{1c} level and higher glycaemic variability affected nitrosative stress and telomere dynamics and triggered early-onset T1D (158). Puberty was linked with physiological insulin resistance and associated weight gain, which was	In type 1 diabetes girls, oral contraceptives were associated with higher cardiovascular risk and worse metabolic control (171).	Anorexia and bulimia were more prevalent in girls with type 1 diabetes, although clinically less serious eating disorders were observed in boys (159).	Attention-deficit hyperactivity disorder (ADHD) was more common in boys with T1D (159).

	more pronounced in girls with T1D (159). Age was shown to have a profound impact on T1D, youth patients were more greatly impacted than adults, with immune interventions exhibiting greater effects in children compared to adults (160). Lifestyle interventions, especially exercise training programs, prevented, delayed or attenuated loss of functional β-cell mass in T1D, in part via activation of the transcription factor STAT3 (partially via IL-6) in β-cells and consequently ERK1/2 activation (47).			
RHAPSODY	**β-cells**		**T2D**	
	A novel pathway to induce β-cell dedifferentiation was suggested through the binding of FGF2 to FGF receptors 1c (FGFR1c), which induced the expression of SOX9, NEUROG3, and gastrin (76).		Amylin and nucleobindin–1 were found to inhibit insulin fibrillation, attenuated fibril-induced cell toxicity, and proposed to act in the control of amyloidogenesis in type 2 diabetes (162).	
BEAT-DKD	**DKD**			
	Initiation: (i) PAN-induced glomerular injury, in adult rats, increased the abundance and activity of YAP and led to the onset of proteinuria, (ii) miR-92a was involved in the deleterious response to immune injury, starting a cascade of podocyte-destabilizing molecular events that led to glomerular destruction, (iii) STAT3 activation was required to trigger miR-92a pathogenic expression, (iv) GSK3 function maintenance in the podocyte to protect against the development of severe kidney disease and (v) conditional depletion/loss of DNA maintenance methyltransferase Dnmt1 in the murine nephron progenitor population led to hypoplastic kidneys, with reduced nephrogenesis and transcriptional changes in the cap mesenchyme population (69,164–166). **Progression:** (i) Podocyte detachment is a key factor of DKD progression, whereas EPB41L5 FA-associated complex and glomerular basement membrane composition contributed to changes in podocyte adhesion signalling, (ii) chronic exposure hypoinsulinaemia promoted podocyte insulin receptor degradation, and (iii) key age-related mechanisms associated with CDK progression were mTOR pathway, the p53 signalling cascade, cell cycle regulation and mechanisms like focal adhesion or insulin resistance (71,167,168).			
4. Develop a platform of pre-clinical assays				
SUMMIT	**Animal modes**			
	DKD	**CVD**	**DR**	
	The more relevant animal models developed were two rat models, the Zucker Diabetic Fatty Rat (ZSF) and the BBDR.CG-lepr.cp (65). A glomerular expression profile database for T1D	The main animal models developed were the Akita mouse model, the SUR1xIGFII and SUR1xLDLR KO mice, BBDR.CG-lepr.cp rat model	The key models developed were the Akita mouse model and a transgenic mouse model with insulin-like growth factor II overexpression (65).	

	and T2D mouse models and at different time points after diabetes onset was generated (65).	and the CUG triplet repeat RNA binding protein 1 transgenic mouse model (65,219,220).	
IMIDIA	**Biobank**	**Animal models**	**Laboratory Protocol**
	The project established a multicentre biobank of human islets and pancreas tissues from organ donors and metabolically phenotype pancreatectomized patients (83). It was produced an EndoC-BH1 cell line that expressed many specific β-cell markers, which was used for laboratory studies of the human β-cells, treatment screening and testing replacement cell therapy in diabetes (79).	It was created a model with pancreatic alpha cell-specific deletion of Tcf7l2, named Tcf7l2AKO mice (80).	It was published a protocol for islets isolation from human pancreatic tissue obtained from type 2 diabetes and non-diabetic patients (78–83).
StemBANNC	**Biobank**	**Laboratory Protocol and Catalogues**	
	By the end of the five-year project, the team had generated 491 quality controlled, well-characterised iPSC lines from 496 people. For diabetes, the main examples are NKX6.1 and MAFA lines. The iPSC lines are accessible to the scientific community through EBiSC and Coriell (148,172,173).	It was developed a protocol for differentiation of endocrine pancreas that enabled the epigenetic and transcriptional profiling of pancreatic differentiation and it was reported the molecular profiling and physiological characterization of some diabetic lines (172,178). Identification and cataloguing of beta-cell-derived iPSCs (BiPSC)-specific differential open chromatin sites (Bi-DOCS) that represented signatures of epigenetic memory and carried the differences in gene expression of endocrine pancreas lineage (176).	
EBiSC	**Biobank**		
	It was created an online catalogue, available at https://cells.ebisc.org/, containing relevant iPSC lines specifics to Diabetes, it has specific iPSC lines to MODY disease and rare diseases, which maximize the potential and the value of this cell bank biobank. Until August, there were available 62 iPSC lines for diabetes mellitus disease and 3 iPSC lines for MODY disease. These 62 iPSC lines derived from patients with monogenic diabetes, patients with early-onset familial type 2 diabetes, Wolfram and patients without the variant disease. 59 iPSC lines of them were developed by StemBANCC and 3 were developed by the University of Newcastle. Concerning MODY iPSC lines, all three were developed by Bioneer. The cells were obtained by healthy donors, and with gene-editing tools obtained mutations related to MODY disease. Two of iPSC lines have HNF1A gene mutation and one has HNF4A mutation. For each of the iPSC lines, EBiSC provided the following information: donor gender, disease status, and variant, if applicable, donor age, depositor, cell line name and alternative names, biosamples ID, hPSCreg name, derivation, culture conditions, characterization and genotyping (174,175).		
INNODIA	**Catalogues**		
	A first catalogue of the HLA-I peptidome of human β-cells, using an immortalized β-cell line naturally expressing the most prevalent HLA-A2 variant (43).		
RHAPSODY	**Laboratory Protocol and Catalogues**		
	It was developed a new protocol for AMPK function study in mouse and human pancreatic islets (179).		

	It was created a catalogue of cis-eQTLs with relevance to T2D and HbA_{1c} levels (177).	
5. Develop systems models		
SUMMIT	Two novels *in silico* models were generated, more precisely (i) Dynamic Bayesian Network model for long-term simulation of clinical complications in type 1 diabetes, and (ii) *In silico* model of aspirin action of platelet and megakaryocyte cyclooxygenase-1 (65).	
BEAT_DKD	*Drosophila* Nephrocyte was proposed as a, well equipped to reveal mechanisms of podocyte function and glomerular diseases (180). There was developed systems biology approaches through the integration of bioinformatics tools to match the patient-specific non-invasive molecular profile with the medicine molecular profile, to better prediction of patient's response for treating DKD (181). It was settled an *in-silico* analysis approach to identify compounds reversing a set of renal age-associated genes significantly associated with DKD progression (71).	
Innovative clinical trial paradigms		
1. Utilise innovative endpoints, trial designs, simulation and analytical approaches		
DIRECT	A novel design model for clinical trials, genotype-based recall (GBR), exhibited to have a higher power to observe gene-drug and gene-lifestyle interactions than same-sized conventional randomized clinical trials, exceptionally when the sampling frame is too small to identify minor alleles and the recalled groups are not sufficiently distinct (182).	
BEAT-DKD	It was proposed a change in the design of studies with medicines to DKD in T2D patients. Those corresponded to directly selecting patients with a positive surrogate marker response to medicine into trials and deselecting patients with a negative surrogate marker response. This claim was supported by two main reasons, firstly the possibility of trials be smaller, shorter, or both, and thus potentially less expensive and secondly the non-responders patients will not be exposed to a medicine that is unlikely to benefit them (183). To help with this issue, it was proposed the concept of "basket" or "umbrella" trials, or more commonly called "platform" trials, that involves assessing the effects of multiple interventions on one or more conditions, using modern adaptive designs and statistical approaches, such as master trial protocol (183,184).	
2. Develop innovative clinical endpoints		
DIRECT	A new prediction model DIRECT-DETECT was developed and validated by this project. This model was based on biomarkers for glycaemic deterioration before and after the onset of type 2 diabetes, including biosamples such as venous blood, faeces, urine and nail clippings characterized at genetic, transcriptomic, metabolomic, proteomic and metagenomic levels. The current prediction model was proposed to be used in the selection process in clinical trials (185,186).	
Innovative Medicines		
1. Identify new or alternative therapeutic concepts (targets) for treatment and prevention of disease		
	T2D and CVD	**T2D and DKD**
SUMMIT	**New targets:** For the treatment of accelerated atherosclerosis in diabetes SUMMIT proposed as a potential target the inhibition of the Nuclear Factor of Activated T-cells signalling (195).	**New targets:** for treating of glomerular disease in T2D patients the pathways proposed were reducing YAP activity, anti-miR-92a options, such as anti-STAT3, partial mTOR inhibition, PI3K-Akt and Raf-MEK-ERK signalling, overexpression of a positive regulator of

		insulin receptor or knockdown a negative regulator (PTP1B), protected podocytes from ER stress and enhancing DCXR expression (69,165,196–198).
IMIDIA	**Diabetes**	
	New targets: activation of glucokinase, a glucose sensor in β-cells, for counteracting hyperglycaemia in individuals with type 2 diabetes (190). **New concepts:** reprogram α-cells to insulin production after extreme β-cell loss (199).	
StemBANNC	**Diabetes**	
	New targets: activation of DYRK1A and overexpression of miR-375 (148–150,187–189). **New concepts:** dedifferentiation and redifferentiation of β-cells by treatment with a redifferentiation cocktail (RC), ARX shRNA, expression of NEUROG3 gene or by controlling/decrease HIF1-α levels (148,150,187,189).	
EMIF	**Diabetes**	**T2D and obesity**
	New targets: modulation of BCAA metabolism (59).	**New targets:** upregulation of adipose tissue CIDEA mRNA levels in T2D patients with obesity (194).
INNODIA	**Diabetes**	**T1D**
	New concepts: dedifferentiation and redifferentiation of β-cells through PolyI:C (193).	**New targets to prevent or reverse β-cell loss:** prevent GRP78 surface translocation or suppress the GRP78 receptor signalling, increase the expression of MANF gene, targeting IFNα and gelofusine of GLP-1R and stimulation of mTORC1 (37,107,154,155,191–193)**.**
RHAPSODY	**Diabetes**	
	New concepts: use of tRNA fragments through epitranscriptome-based therapy by RHAPSODY (200).	
2. Develop novel therapeutic agents and disease prevention strategies		
SUMMIT	**T2D and CVD**	**T2D and DKD**
	Aleglitazar is a dual peroxisome proliferator-activated receptor-α/γ agonist which were under development by Hoffmann–La Roche for the treatment of type II diabetes but its development was halted and abandoned phase 3 trials due since the medicine caused fractures, kidney failure and heart failure and showed lack of efficacy (201,221). For patients with diabetes, a low-dose aspirin for the secondary prevention of cerebro-cardiovascular events as atherothrombotic vascular complications in patients with diabetes showed to be effective with favourable benefit/risk ratio, however, this benefit was not exhibited for primary prevention (203).	Renin-angiotensin-aldosterone-system inhibitors which clinical trials evidenced that slowed the onset of T2D and reduced the risk of renal complications in manifest T2D (202).
DIRECT	**T2D**	
	Data supported that metformin may provide cardio-metabolic benefit, even in a non-diabetic population and beyond reducing the risk of developing T2D (204).	
StemBANCC	**Diabetes**	
	There were established four stem cell-based replacement treatments: (i) Promoting regeneration in situ by endogenous stem cells, (ii) Regeneration in situ by application of exogenous stem cells, (iii) Repopulating decellularized donor kidney with a patient's stem cells, and (iv) Construction of entirely new transplantable kidneys, with	

	two broad techniques: precision engineering by positioning everything exactly, for example, by 3D printing, or by supporting cells' self-organizing ability (207–209).
BEAT-DKD	**Diabetic nephropathy**
	SGLT2 inhibitors and GLP1R agonists showed clinical efficacy in the treatment of diabetic nephropathy. SGLT2 inhibitors (such as canagliflozin) slowed the progression of DKD and GLP1R agonists prevented worsening of albuminuria (205,206).
3. Implement new approaches for the development and production of biopharmaceuticals and tissue engineering	
StemBANCC	For the clinical translation of human pluripotent stem cells (hPSCs), in diabetes research and industrial field, continuous peristaltic pump-based circulation technology, in a hydraulically driven bioreactor, with suspension culture of hPSCs, showed to be a potential 3D tool for large-scale production (210).
Patient tailored adherence programmes	
1. Develop and apply patient-centred predictive models	
DIRECT	The two new models of disease architecture were (i) the 'Palette' model, centred on a molecular taxonomy, which contributes to diabetes risk and progression, in opposition to the 'pigeonhole' that is based on 'You have type 2F diabetes; your diabetes is caused by a defect in x and the correct treatment is y', and (ii) the DIRECT-DETECT prediction model based on biomarkers for glycaemic deterioration aimed at identifying non-diabetic individuals who are at high risk of rapid, short-term glycaemic deterioration and predict new-onset diabetes glycaemic deterioration in people who have recently been diagnosed with type 2 diabetes (185,186,211).