Novel immunotherapies for type 1 diabetes

Reporting

Project Information

NAIMIT
Grant agreement ID: 241447
Funded under FP7-HEALTH

Project website

Status
Closed project

Overall budget € 14 247 400
EU contribution € 10 920 800

Start date 1 November 2009
End date 30 April 2015

Coordinated by KATHOLIEKE UNIVERSITEIT LEUVEN Belgium

This project is featured in...

RESEARCH*EU MAGAZINE
Dementia: investing against the trillion dollar disease

Final Report Summary - NAIMIT (Novel immunotherapies for type 1 diabetes.)
Executive Summary:
The concept that was the basis for the NAIMIT project, namely that the beta-cell and the immune system are partners in crime in the pathogenesis of type 1 diabetes, has since the beginning of the project received a lot of support worldwide and at the present day, the central role of the beta-cell in its own destruction is accepted by the scientific community. The NAIMIT project and the members of the consortium have contributed in a significant way to the elaboration and validation of this concept. The steps taken towards finding a cure or preventive interventions allowing to arrest type 1 diabetes have been significant and almost all workpackages (WP) have reached their projected endpoints, with promising interventions on the verge of clinical application or up-scaling towards clinical application. Our bold projection of exploiting natural immune modulators and introducing pathways towards individualization of therapy helped by pharmacogenetics has proven to be a realistic one.

In WP1, we have demonstrated that the projected effects of 1α,25-dihydroxyvitamin D3 treatment (ex vivo) of dendritic cells (DCs) of healthy people and type 1 diabetic patients leads to the generation of a tolerogenic DC phenotype. This phenotype is robust and is able to induce tolerance in the T cell compartment and educate other DCs. These vitamin D-induced tolerogenic DCs were evaluated in vitro and in animal models of type 1 diabetes within the NAIMIT consortium and approval for a phase I clinical trial has been obtained for which we are in the process of recruiting patients. This phase I trial aims to test the stability of the tolerogenic DCs upon re-introduction in patients with type 1 diabetes and establish the safety of the intervention. A crucial role in the realization of this part of NAIMIT was played by the SME Dandrit in the first year of NAIMIT, establishing standard operating procedures (SOPs), but at the end of year 1 Dandrit left the consortium and all work was taken over by LUMC, where also the clinical trials are running.

In WP2, the path of tolerance induction through cutaneous administration of type 1 diabetes-relevant peptides was pursued, with introduction of 1α,25-dihydroxyvitamin D3 and corticosteroids as immunomodulators applied topically. Within NAIMIT the crucial animal (mouse) model for preclinical testing before introducing the multi-peptide approach in patients was developed through collaboration between several partners within the consortium. The intervention with single peptides has been optimized and patients for clinical trials are being recruited.

In WP3, a brand new technology using soluble T cell receptor (TCR) technology has been explored. This technology has been developed by Immunocore, one of the NAIMIT SMEs, and very intense work on translating the TCR technology from the cancer field to the autoimmunity field has yielded crucial knowledge: through collaborations between different NAIMIT partners, we gained insight in the way autoantigens bind to the Major Histocompatibility Complex proteins on the cell surface of DCs and the TCR. The aberrant binding pattern of autoantigens (inherent to the disease) renders the development of the TCR-based technology for autoimmune diseases difficult, but not impossible. The status at the end of NAIMIT is that the SME has pursued its work in the cancer field (and has been rewarded with many collaborations), but has decided to also pursue the newly discovered exploration paths in type 1 diabetes after NAIMIT has ended.

WP4 aimed to explore the potential of introducing (auto-)antigens via the gut or modulating the gut immune system with probiotics in order to induce tolerance in type 1 diabetes. In this WP most efforts were put in the project where type 1 diabetes-relevant antigens, such as proinsulin, GAD and IA2 were introduced in the gut, via the Lactococcus lactis platform, developed by the SME Actogenix. By this technique diabetes could be reverted in NOD mice and at this moment clinical trials are being considered.
The role of the beta-cell in its own destruction was elaborated in WP5, with discovery of novel pathways involved in beta-cell destruction, beta-cell defense but also the interaction between the beta-cell and the immune system. Several new type 1 diabetes candidate genes, expressed in beta-cells, were identified. Furthermore, the pathway of novel antigen generation through alternative splicing and posttranslational modification of beta-cell proteins was investigated, with identification of new auto-antigens.

In WP6 we were able to identify polymorphisms in the pathways involved in vitamin D metabolism that were contributing to the responses of DCs and T lymphocytes to 1α,25-dihydroxyvitamin D3. This was achieved via analysis of DCs and T cells used in WP1 and 2 and opens novels pathways for patient stratification in the interventions using vitamin D, thus leading to the promise of individualized therapies. Major emphasis was put on training in NAIMIT and WP7 contributed to initiatives on education, but mainly focused on facilitating the frequent exchanges of people and methods between labs.

Finally, in WP8 the coordination of NAIMIT happened, overall leading to a smooth and fruitful project.

Project Context and Objectives:
Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterised by immune-mediated destruction of insulin-producing beta-cells in the pancreas. The incidence of T1DM in childhood is increasing at about 3% per year, equivalent to a doubling time of 20-25 years. T1DM is the most common metabolic disease in the young. In addition, 5-10% of patients originally diagnosed as T2DM have a less severe and more slowly progressing form of T1DM, usually referred to as “Latent Autoimmune Diabetes of Adults” (LADA). Thus, collectively about two million people in Europe (around 0.5% of the population) and several million people world-wide suffer from this disease. Of concern are the reports of a steady increase of T1DM over the last decades in virtually all European countries and a particular escalation in the 1-5-year age group. Since it carries a significant chronic disease burden, T1DM has thus become a major public health concern worldwide, emphasizing the urgent need for safe and effective intervention and prevention strategies.

At the same time that this gloomy picture has emerged, a series of key basic and clinical scientific advances have promoted the concept that the pathological autoimmunity characteristic of T1DM is potentially reversible through subtle modulation of the immune system. Pioneered in animal models, encouraging preliminary data along these lines are also beginning to emerge in man. These approaches are characterised by the fact that they aim to exploit natural routes towards beta-cell protection and restoration; in doing so they offer a real prospect of reversing autoimmunity and restoring tolerance to beta-cells. These approaches were pursued in within NAIMIT and are in contrast with those that aim to inhibit autoimmune responses using immunosuppressive protocols, such as cytostatic agents and monoclonal antibodies that deplete or modify selected lymphocyte subsets. Although such short-term immunosuppression appears capable of beta-cell preservation, the effects are not sustained and there is no evidence to date that beta-cell tolerance is induced. Moreover, many such interventions to date have been accompanied by considerable side effects. Our proposal embodied a further concept, not always given due recognition, namely that “one treatment does not fit all”. T1DM is a complex polygenic disorder with clinical and, probably, pathogenetic heterogeneity. It is apparent, for example, that not all patients exhibit polymorphisms in the same predisposing genes. Thus, it is likely that there are multiple routes by which beta-cell tolerance is broken and more than one route by which it can be restored, which may vary from patient to patient. As an example, patients could differ in their responsiveness to antigen-specific immunotherapy with insulin, modulation of regulatory T-cells (Tregs) or treatment with vitamin D-related compounds, according to their possession of polymorphisms in genes such as INS, IL-2RA or those...
related to vitamin D metabolism, respectively. Inclusion of this pharmacogenetic theme within NAIMIT was a major innovation.

The goals of a successful therapeutic approach to type 1 diabetes (T1D) are cessation of beta-cell destruction, reversal of autoimmunity and preservation of surviving beta-cells allowing any natural regenerative potential to be realized. These are crucial challenges for diabetes research. Any such interventions to achieve beta-cell protection and restoration should realize these goals through modulation of the immune system of the patient to a minimal degree in order to avoid severe disturbances of immune surveillance mechanisms leading to intolerable side effects. The present state of our understanding of the pathogenesis of T1D indicates that, in principle, therapeutic success is achievable and that interference in the progressive loss of beta-cell mass in newly diagnosed T1D patients is within reach. This required innovative approaches that operate with a minimal degree of interference in the general functions of the immune system. In order to move the field forward in this respect, we proposed a series of studies representing a novel and integrated approach. Our studies, organized into six scientific work packages, plus a training and an administrative work package, were designed as overlapping and complementary strategies that run the full-length of the journey from bench to bedside, including innovative first-in-man studies that aim to arrest autoimmunity in T1D with minimal or no immune suppression; rather we aimed to harness its extraordinary natural power for regulation, healing and regeneration. The underlying concept for the therapeutic interventions developed in this proposal is the central role of the immune system in T1D, but we also recognized the important role that the beta-cell adopts, as a partner actively contributing to its own demise. This needs undoubtedly be taken into account when designing strategies for therapeutic interventions for beta-cell protection and restoration. The concept of a key role for both the immune system and the beta-cell highlights the need for a “multiple hit” approach to disease prevention. This includes both modulation and re-education of the immune system, boosting of beta-cell defenses against autoimmune damage and arresting the pro-inflammatory dialogue between immune cells and beta-cells.

We proposed an original concept, in which natural immune modulators were introduced in interventional approaches that modify the immune system through an antigen-specific route to achieve beta-cell protection. Only by intervening in an antigen-specific way can one guarantee beneficial immunomodulation with minimal unwanted immune perturbation in the quest to reverse autoimmunity. As stated above, a major emphasis was placed on the integration of knowledge on the understanding of how the beta-cell is destroyed in order to arrive at interventive therapies that protect and restore beta-cell function. Moreover, we have included in our consortium leading researchers in immunogenetics, opening up the prospect of individualised therapeutic interventions tailored to polymorphic components of the adaptive and innate immune response. This contrasts with the conventional monotherapies widely considered by other scientists, which disregard important inter-individual variations in the disease process. As an example, the genetic expertise in our consortium enabled us to make and test predictions of responsiveness of certain receptor genotypes or polymorphisms to the products to be examined, such as vitamin D or glucocorticoids. To achieve these objectives, we have assembled a consortium of highly-renowned scientists with special expertise in the fields of immune- and pharmaco-genetics, basic and applied immunology, beta-cell pathophysiology, translational models and clinical diabetes, to drive the process of discovering cures for T1DM.

We have based our approach on the following pathophysiological model (see Fig. 1). After beta-cell damage, for instance due to a metabolic assault or a viral infection, beta-cell antigens are released and picked up by antigen presenting cells (APC), typically DCs, that in turn will recruit and activate T
lymphocytes. The APC is the key cell in determining the direction of the immune response, since it produces essential signalling molecules, such as cytokines and chemokines, under the influence of which T lymphocytes are activated and polarised. Once T lymphocytes have entered the scene, the immune response starts to spread, with involvement of more immune cells, such as macrophages and activated T lymphocytes. This consortium of immune cells will damage the beta-cells using different attack strategies, such as direct CD8+ T-cell-mediated killing via perforin/granzyme B or cytokines and free oxygen and nitrogen radicals. For long it has been assumed that the beta-cell is an innocent victim of this process, but it is increasingly evident that it actively contributes to its own demise, since in response to the immune attack and increasing stress of controlling blood glucose, it up-regulates autoantigen expression and releases crucial immune molecules, such as chemokines, further shaping the immune reaction. In this model, there are several checkpoints that have been targeted in our intervention studies:

1. The antigen-presenting DCs
2. Autoreactive T lymphocytes
3. The beta-cell as active partner in its own demise

The specific S&T objectives of the project NAIMIT were:

1. To explore novel immunomodulatory approaches using natural immunomodulators (e.g. antigen, glucocorticoids and vitamin D derivatives) to modulate dendritic cell (DC) and T-lymphocyte function, both in vitro and in vivo with the aim of inducing antigen-specific regulation.
2. To introduce novel immunomodulatory tools to induce antigen-specific tolerance: soluble monoclonal TCRs (mTCRs) and Lactococcus lactis (L. lactis).
3. To gain insight into the mechanisms involved in immune-mediated beta-cell death aimed at harnessing the beta-cell against autoimmune attack.
4. To unravel the means of communication between beta-cells and the immune system in order to interfere with beta-cell destruction and propagation of the beta-cell destruction process.
5. To explore genetic variants responsible for the response to interventions allowing individualized therapies.
6. To build a consortium spanning basic and clinical research to allow new and individualized therapeutic approaches for clinical interventions in T1D in the future.

Project Results:
In this part we describe the main results obtained during the 5.5 years of the NAIMIT project, divided into the different work packages. In relation to the specific objectives set forward at the start of the project, we are happy to report that almost all milestones and deliverables have been achieved (except for 1 out of 61 deliverables and 1 out of 46 milestones). This near-complete success was in part made possible through the granting of a 6 month extension by the European Commission (see table of deliverables attached). Thus, the level of advance was achieved as predicted, reaching deliverables and milestones on or even ahead of time. Close monitoring of progression of the work by the coordinating team has been in place. The number of publications published by the consortium has increased every year, with 11, 30, 40, 44 and 51 publications in peer-reviewed journals, respectively (see table of publications attached). Of note, especially also collaborative publications between beneficiaries of the consortium have steadily increased in number over the 5.5 years duration of the project.

WP1 – Re-educating antigen-presenting cells
The work in WP1 was designed to interfere with antigen-specific autoimmunity but to have minimal impact on the global immune system. In this “bench to clinical assessment” work package, autologous DCs were rendered tolerance-inducing in an antigen-specific manner. It was the aim to modulate DCs ex vivo towards a tolerogenic phenotype using “natural” mediators (namely active vitamin D (1,25(OH)2D3 - VitD) and/or glucocorticoids (GCs)). Tolerogenic DCs were being manipulated to orchestrate beta-cell-specific tolerance, by pulsing them with beta-cell auto-antigenic peptide epitopes, such as proinsulin C19-A3. The work package has achieved favorable results in respect to all objectives. We have elaborated on the mechanistic characteristics of the DCs generated in the presence of 1,25(OH)2D3 – VitD, using in vitro models, as well as studying the effect of these DCs on T lymphocytes. As such, we demonstrated that different types of Treg cells are being induced. Intense collaborations on comparing preclinical and clinical findings demonstrated the major difference between the nature of human and murine DC and their responses to VitD, as well as their characteristics needed for tolerogenicity. Data are now available on 3 different mouse models: NOD mice, RIP-LCMV mice and HLA-DR4 Tg mice (see also WP2). Migration studies in the mouse models revealed a targeted migration of tolerogenic DCs towards mouse pancreas and pancreas-draining lymph nodes, compared to untreated DCs, provided that they are injected s.c. ventrally. In vivo capacity of modulated DCs to induce pro-insulin specific regulatory T cells was demonstrated in humanized HLA-DR4-Tg mouse, and the capacity to reduce diabetes incidence in transgenic RIP-LCMV mice, which provided a proof-of-concept for in vivo induced immune regulation using tolerogenic DCs.

Besides this basic scientific work, most efforts were put on translating the knowledge to clinic, allowing as soon as possible the introduction of this interesting tool into patients. For this, GMP-grade media and supplements have been introduced in the protocols, SOPs validated, thus upgrading the tolerogenic DCs to a GMP cell product. Also we gathered data showing that tolerogenic DCs can be induced from monocytes of T1D patients. A clinically approved modulation/maturation protocol was selected and further tested using repeated stimulation with infectious and inflammatory agents. This confirmed stable tolerogenic phenotype and function of the clinical-grade tolerogenic DCs. GMP-grade media and supplements have been incorporated and validated and tolerogenic DCs have been upgraded to a GMP cell product. Also, we defined clinical release-criteria for functional tolerogenic DCs and the large-scale GMP-grade generation protocol, which were validated in three test-runs. The clinical-grade tolerogenic DCs generated in the test runs fulfilled all release criteria for clinical administration. We also confirmed that tolerogenic DCs, which fulfill clinical-release criteria, are generated from monocytes of type 1 diabetes patients. Finally, as a cherry on the cake, we have recently obtained approval from the National Central Committee on Research Involving Human Subjects (CCMO) to test safety and feasibility of tolerogenic DC therapy in humans. At present, patients are being recruited for performing this Phase I clinical trial.

WP2 – Restoring the T-cell balance
This WP aimed to restore the T-cell balance through an antigen-specific route that avoids global immune suppression. It build upon the emerging program of Peptide Immunotherapy (PIT), in which naturally processed and presented peptide fragments from major beta-cell auto-antigens are administered intradermally to promote the generation of islet-specific Tregs (IS-Tregs). Use of multiple peptides to enhance the power and breadth of the approach is a major asset. Furthermore, topical adjuvants such as VitD, retinoic acid and GCs have been studied for their potential to enhance PIT. Significant progress has been made on defining the changes in DCs in human skin following topical treatment with the VitD analogue, calcipotriol alone or in combination with steroid (betamethasone) or a
derivative of vitamin A (retinoic acid). Topical treatment with betamethasone or betamethasone plus calcipotriol reduces levels of proinflammatory cytokines in interstitial fluid with relative preservation of the regulatory (potential tolerogenic) cytokine IL-10. Further, 9 peptides from IA-2 and proinsulin identified by biochemical and in vitro testing have been determined as candidates for development in a Multi-Pep cocktail. These have been synthesized in large scale and have been combined at operational concentrations without precipitation. Building on NAIMIT, additional extra-mural funding (Welcome Trust) has been obtained via a competitive award for the chemical and toxicological testing of the cocktail, as well as GMP grade synthesis; through to preparation for a Phase I study. Work commenced in 2012 on method development for: GMP-grade and clinical scale of synthesis; solubility analyses for the 9 candidates; and method development for identification and progress to date has been excellent. The final selection of peptides for the MultiPepT1D cocktail has been made (Q2/3 2013) and: (i) a phase appropriate method set has been developed for peptide measurement, monitoring and stability; (ii) sterile non-GMP cocktail has been syntheised, combined, sterilized and distributed to contract research organizations for toxicology; and (iii) full GMP synthesis has been completed; (iv) phase-appropriate in vitro and in vivo toxicology has been completed; (v) all required regulatory approvals are in place to commence a Phase I study focused on safety; (vi) extra-mural funding has been obtained to support this Phase I study which will start in Q2 2015.

In preclinical models tolerance studies centred around these peptide cocktails have become further established. As such, a preclinical model has been established, in which HLA-DR4 Tg mice were rendered “autoimmune” by immunization with proinsulin. The autoimmunity in this mouse model could be prevented by pre-treatment with single proinsulin peptide immunotherapy. Existing autoimmunity could also be suppressed by administering cocktails of the same human peptides that are under clinical evaluation. These effects were associated with expansion of proliferating FoxP3+ regulatory T-cells and IL-10 conversion. We have used this model to study dosing (low dose is optimal) and frequency (multiple administrations are required). Further, requirement for thymus-derived regulatory T-cell induction in the process of peptide immunotherapy has been demonstrated.

Finally, an extensive and operational clinical network of biological samples from new-onset cases of T1D has been established for preclinical and clinical intervention studies, in order to prepare eventual interventions and proof of concept in these patients.

WP3 – TCR-directed immunotherapy

In this WP the body’s own system of recognizing antigens on the surface of cells, namely the T-cell receptor (TCR), has been exploited by a very innovative technology, developed by the SME partner Immunocore. Soluble monoclonal TCRs (mTCRs) directed against specific beta-cell antigen epitopes in the context of the common HLA-A2 (A*0201) molecule, an allele present in the majority of T1D patients, or its mouse equivalent, have been engineered. These allow target-specific delivery of therapeutic agents. Different pathways have been taken: first linking mTCRs directed against beta-cell specific antigens specific for the mouse system have been linked to IL4, IL13 and IL10 in order to perform proof of concept trials in preclinical mouse models (NOD). These mTCRs have been synthesized and the first experiments in mice using IL4 and IL13 fusions have been completed; IL10 constructs were too low in activity to merit in vivo testing in mouse models and have thus been discontinued. Data on IL4 and IL13 were disappointing as treatment with these TCRs could not delay diabetes recurrence in NOD mice. Second, major mTCR engineering and intense collaborations between different NAIMIT beneficiaries have allowed SME Immunocore to go in depth into the synthesis of human mTCRs directed against beta-cell specific antigens specific for the human system. Several clonal mTCRs have been synthesized and their effect on diabetes in NOD mice generated, with some mTCRs being promising to become a lead candidate. Further experiments are in progress to find the optimal construct and derive the mTCR cocktail to be used in the next phase of clinical research.
autoantigens. It has become evident that all autoantigens identified until now, have very low binding affinity, thus making easy synthesis of mTCRs a challenge. The 4th year of NAIMIT has been successful for engineering of the mTCR specific for pre-proinsulin, overcoming significant technical barriers due to the low affinity of the wild type receptor; this was achieved in part as a result of targeting mutations to the regions of the TCR making direct contact with the target MHC-peptide, identified from a high resolution crystal structure and additionally elongating other regions to bring them into contact with the MHC-peptide. Recently, pico-Molar affinity was achieved, with a binding half-life of over 14 hrs, which is a significant achievement for a sub-optimal autoimmune TCR. Finally, exploration of linkage of mTCRs to other immunomodulators that are even more interesting in the context of type 1 diabetes, as alternative effector mechanisms to IL4 and IL13, have been investigated, namely fusion of TCRs to scFv antibody domains which induce an inhibitory immune response. Thus this tool is ready for linking to eg. imaging molecules and will be explored as described in the aims of the WP for imaging of human beta-cells. This achievement could be realized through an intense collaboration between in particular Immunocore and WP2. In this regard, promising in vitro data has been obtained i.e. inhibition of T cell proliferation in response to TCR-scFv fusion proteins bound specifically to target MHC-peptide complexes on APCs.

WP4 – Mucosal intervention for tolerance restoration
In this work package focus was put on induction of mucosa-mediated tolerance to islet antigens. Orally administered antigen encounter the gut associated lymphoid tissue (GALT), a well-developed immune network that not only evolved to protect the host from ingested pathogens, but also developed the property of preventing the host from reacting to ingested proteins. Modulation of immune-responses in GALT has been shown to be relevant to prevent/delay autoimmune diabetes onset. Two distinct paths have been followed and we have obtained promising results in the two research branches of this WP. In the first branch, the potential of probiotics administration as immunomodulator in T1D has been explored. We have performed in vitro experiments aimed at characterizing the immunomodulatory effects of different probiotic bacterial strains. In vivo studies in NOD mice showed that probiotic treatment significantly decreases islet expression of proinflammatory cytokines and chemokines and, in parallel, determines an increased islet IL10 expression.

Second, we have explored the therapeutic potential of an original tool introduced by beneficiary 12 (SME ActoGeniX), in which recombinant Lactococcus lactis (L. lactis) (ActoBiotics™) is a carrier for peptides, in association with immunomodulatory molecules, allowing delivery of antigen to the GALT. Extensive experiments using L. lactis expressing human proinsulin in conjunction with IL10 have been carried out. These studies demonstrated that reversal of diabetes in newly diagnosed diabetic NOD mice can be reached in over 60% of cases, when administered in combination with low, sub-therapeutic doses of anti-CD3. This therapeutic effect is combined with the induction of CD4+CD25+FoxP3+ regulatory T cells in the pancreatic draining lymph nodes. Mechanistic studies on this regulatory T cell population highlighted their capacity of delaying diabetes, as shown by co-transfer studies. Regulatory T cells were shown to migrate, accumulate and proliferate locally in the islets upon combination therapy and suppress effector T cell responses in an antigen-specific way. By using genetically manipulated models (FoxP3-DTR NOD mice), we could demonstrate that eliminating the FoxP3+ T cells, broke the tolerance installed by the ActoBiotics™ therapy. We hypothesize that antigen-specific tolerance initiates in the intestinal immune system and then spreads to the periphery by T cells disseminating into pancreatic lymph nodes. More recently, we demonstrated also that administration of human GAD65 or IA-2 along with IL10 was able to reverse diabetes in 56% of newly diagnosed diabetic NOD mice under low-dose anti-CD3 induction.
therapy. Of interest, whereas FoxP3+ Tregs could also be demonstrated in cured mice, these were not present in pancreas, but remained in draining pancreatic lymph nodes, suggesting a different process from the proinsulin-based therapy. Of interest, when a similar therapeutic set-up was used (ActoBiotics™ therapy combined with low dose anti-CD3) using an irrelevant antigen, like ovalbumin, or a mosaic protein comprising fragments of 3 beta-cell specific antigens (GAD65, IA2 and proinsulin) no reversal of recent onset diabetes could be obtained, demonstrating the specificity of the therapy.

Clinical grade strains of L. lactis (stably genomically integrated constructs instead of plasmid-driven) have been prepared by ActoGeniX and have been tested in NOD mice. Exactly the same disease protection as with the plasmid constructs has been demonstrated. Finally, laser-capture microdissection (LCM) of pancreatic islets (endocrine portion and insulitis separately) from both cured and non-cured combination-treated animals allowed analysis of mRNA and miRNA patterns. At the same time, plasma samples were taken for miRNA analyses and the obtained data show major alterations in the pancreas and peripheral blood of mice treated with the ActoBiotics™ therapy. In the meantime, intense negotiations on the design of a clinical trial in humans with type 1 diabetes have been ongoing between ActoGeniX and different industrial partners as well as international regulators. Partially thanks to the positive results obtained in preclinical models of type 1 diabetes, ActoGeniX has perspectives of integration in a bigger pharmaceutical entity at the end of NAIMIT.

WP5 – Beta-cell protection and restoration: Dialogue with the immune system

In this distinctive work package, we focused on the role of the beta-cell in its own destruction and specifically on the way in which the immune system and the beta-cell communicate.

We have obtained significant insights in the gene networks induced by IL1β and TNFα as well as genes downstream of STAT-1, Jun-B and IRF-1 using microarray and proteomic techniques in mouse and rat models for T1D and by RNA sequencing of human islets. Also in human islets we have performed proteomic 2D-DIGE analysis, to investigate the pathways involved in cytokine-induced beta-cell death as well as the anti-inflammatory role of GLP-1 thereon. These experiments showed an important regulation of REG1 proteins by cytokines, which is normalized by GLP1. A role for several candidate genes for T1D, namely PTPN2, MDA-5, Bach2, TYK2 and Glis3, in beta-cell responses to viral infections and pro-inflammatory cytokines has been identified. These experiments allowed us to identify the specific beta-cell apoptosis pathways triggered following inhibition of PTPN2 or during a viral infection, namely activation of the BH3-only protein BIM. This was the first evidence that candidate genes for T1D may act at the beta-cell level, modulating both beta-cell apoptosis and the virus-induced dialogue between beta-cells and the immune system. Recent observations, obtained by RNA sequencing, indicated that >80% of the known candidate genes for T1D are expressed in human islets and have detectable changes in expression following exposure to pro-inflammatory cytokines. We have recently investigated the role for alternative splicing for inflammation-induced beta-cell apoptosis, and the role for diabetes candidate genes in this process, and observed that the candidate gene Glis3 modulates beta-cell apoptosis via splicing of Bim and that the splicing regulator Nova1, previously considered to be brain specific, plays a major role in the regulation of splicing in beta-cells.

The impact of 1,25-dihydroxyvitamin D3 on control C57Bl6 mouse islets, exposed to inflammatory cytokines (IL1β and IFN, has been studied by microarray analysis. A major effect was seen on chemokine and cytokine expression, regulated via modulation of the key pro-inflammatory transcription factor NF-κB. Finally, experiments in a rat model for type 1 diabetes (LEW.1AR1-iddm) have been performed and many new tools (miRNAs) have been designed allowing better analysis of the dialogue between the beta cell...
and the immune system. Collaborative work inside NAIMIT, involving WPs 2 and 5 has identified a novel role for the Th17 cells and the cytokine IL-17 in the dialogue between beta-cells and the immune system, which contributes to trigger insulitis and beta-cell loss. This involves both IL-17-induced increase in beta-cell apoptosis and augmented local production of chemokines, leading to increased attraction of immune cells to the islets. This provides a major step forward in understanding the role for IL-17 in diabetes, and opens the door for novel therapies targeting IL-17 in order to prevent or revert T1D.

Another important finding was the clarification of the dialogue between ER stress and inflammation in the triggering/amplification of insulitis in T1D. We have shown that ER stress markers are present in islets from patients with T1D, that ER stress amplifies islet inflammation and beta-cell death, and, of particular relevance, that modulation of ER stress prevents diabetes in two mouse models of diabetes. This opens the way for the use of ER stress modulators – one of them (TUDCA) is already in clinical use for liver diseases – as an adjunct therapy to immunomodulation in the early stages of T1D.

In recent years, attention has also gone to the role of ER-stress and inflammation in the induction of posttranslationally modified proteins, that may play a role as neo-autoantigens in type 1 diabetes. As such, post-translational modification of proteins in situations of inflammatory attack on the beta-cell, is a possible way of creating neo-antigens and has been studied intensively, as this could again be a way the beta-cell and the immune system enter into a dialogue. In this regard, we have shown that the abundant ER chaperone Glucose regulated protein 78 (GRP78) is citrullinated. Further analysis of the role of citrullinated GRP78 in the diabetes-prone NOD mice indicated that these mice have autoantibodies and effector T cells that react against citrullinated GRP78, suggesting that inflammation-induced citrullination, specifically in beta-cells, generates a novel autoantigen. These are highly relevant findings, suggesting for the first time a clear role for protein citrullination in T1D, and, more important, identifying the specific protein that is citrullinated.

WP6 – Pharmacogenetics: towards individualized therapies
This WP is built on the hypothesis that interventions should be individualized and tailored to the genetic footprint of the disease in any individual patient. The purpose is to link up with WP1 and WP2, to explore possible genetic signatures to predict responses of DCs and T-cells to VitD and GCs, depending on the presence of polymorphisms in crucial genes in the signal transduction and metabolism of these steroids.

During the first year of NAIMIT focus for this WP was concentrated on organizing the logistic network between the partners involved in WP6 and in establishing SOPs on how to proceed with optimal sample collection, in order to provide samples to the ‘genetics’ partners of every patient and control donor where DCs and T cells are being isolated. SOPs were established and major efforts on minimizing blood volume were made. During year two and three different batches of blood samples from healthy controls and T1D patients have been genotyped for HLA, Vitamin D and GC polymorphisms. The results have been linked to functional DC and T-cell parameters.

In the meantime, further characterization of the vitamin D related genes that are involved in DC and T cell behavior have been studied with results for vitamin D pathway gene expression analysis of lymphocytes from healthy controls in relation to genotypes. In year 4 the first data on polymorphisms on genes involved in the VitD metabolism, transport or action have emerged and to our greatest satisfaction patterns of associations became apparent. During the last NAIMIT year we have extended these observations and used pharmacogenetic data from an interventional trial to identify decision trees clearly dissecting the dose response to vitamin D. Of interest, the same polymorphisms have emerged both in DC and in T lymphocytes predicting effects of VitD, thus opening truly the possibility of personalized medicine and
selection of those individuals who may benefit most or not at all from a treatment involving vitamin D.

WP7 – Training activities in the Collaborative Project (CP)

Four formal training sessions have been organized in conjunction with the annual meetings, and many exchanges took place between partners, with particular attention to the establishment of common techniques and protocols. In the whole consortium the same type of beta-cell line has been used and joint preclinical models and in vitro protocols have been developed. Histological analysis were performed in the same way, guaranteed by exchanges of personnel and protocols. Also many young PhD students and post-doctoral researchers were invited to partner laboratories for training in particular techniques. Thus, an open collaborative atmosphere has been created within NAIMIT, with many formal and informal exchanges between partners and travel of young investigators for short periods of time. Sharing of techniques between partners has resulted in a major learning experience for all young investigators involved in partner labs. Exchanges of models and researchers is still happening and young researchers are shared between labs.

Formal training sessions were also organized for the members of the whole consortium, in particular targeting the young investigators and PhD students, at the occasion of the yearly meetings, discussing topics like ethical use of animal models and appropriate clinical trial design. In addition educational sessions were organized in smaller groups, between partners on topics of collaboration e.g. role of ER stress and in vitro techniques for evaluation of ER stress in beta-cell models.

WP8 – CP management and administration

Management of the project was kept as lean as possible and overall the project ran very smoothly, with major focus on promoting interactions between partners and follow-up of the work. Great effort was put in getting the website (www.naimit.eu) up and running, which was successfully achieved by month 1. This website has been kept up to date and also monitored intensively, in order to promote the work performed within NAIMIT, but also to give internal feedback to partners on where in the world researchers are accessing our website. The website was promoted on a national level in the individual countries whenever activities of NAIMIT happened or e.g. at the occasion of World Diabetesday.

A strict communication plan within the consortium was organized, in particular during year 1 and 2 to promote the spirit of partnership and collaboration in NAMIT. This policy allowed intense communication between partners, with frequent phone-conferences and one-on-one meetings between partners. Formal monthly phone conferences within WPs were organized to get the work going, as well as formal 3-monthly and later on 6-monthly steering committee phone conferences. Particular attention has been put on face-to-face meetings between partners, but in order to save money, often these face-to-face meetings were combined with international conferences where several partners were present (such as the European Association for the Study of Diabetes (EASD) annual meeting and the Immunology of Diabetes Society (IDS) meeting).

Administrative issues were few and when problems arose, they were resolved smoothly through intervention of the coordinating team, mostly via direct contact between the administrative coordinator and the financial officers or local investigators. During the first year, one researcher changed institutions, necessitating the introduction of a new partner institution (Cardiff University). In year two, one SME partner (Dandrit) could not stay in the consortium, as deliverables and milestones could not be reached. In agreement with this partner, the NAIMIT consortium decided to discontinue this partnership. In full agreement within the consortium, very constructive efforts were made to divide the planned work of this
beneficiary within the already existing beneficiaries. In addition, a new scientific team leader from LUMC with specific expertise allowing to reach the proposed deliverables and milestones was integrated in NAIMIT, thus avoiding any impact of the departure of Dandrit on the experimental work. Intensive guidance and interactions with the project officer were essential in realizing this smooth transition. The other 2 SME’s thrived very well, and in part based on the success within NAIMIT, were able to attract new additional funding and significantly extended their autoimmunity core. In year 4 no major managerial issues arose and major attention could go to communication and dissemination. As such, NAIMIT participated to the Open Door Day of the European Commission in Brussels (May 2013) and NAIMIT was invited to present ‘How to write and run an EC project successfully’ at the occasion of the Infoday for the launch of Horizon 2020 (Nov 2013), the new funding scheme of the EC. On the website of NAIMIT, short presentations of the work within NAIMIT have been posted (global overview, SME presentations, partners etc.). NAIMIT further participated in national public initiatives in different countries. Several disseminating articles were written for a general non-scientific public, including civil people, nurses, doctors, and patients. Intense contacts were also kept with JDRF, in lobbying together at the heart of the EC for attention for type 1 diabetes research within Horizon 2020 and IMI2. Throughout the NAIMIT project, the consortium worked together very intensely, in full harmony, not only between scientists, but also between administrators and financial officers. The firm guidance in our effort by the scientific officers assigned to NAIMIT were key in this success.

Potential Impact:
The potential impact of the present project is great, both on a scientific and a therapeutic level. We have managed to execute this work with great energy, with all partners contributing substantially to the goals of the project. The cell therapies, the antigen based approaches, the exploitation of the natural immunomodulators and of great interest, the introduction of the new tools for tolerance induction, all hold great promise. Research has progressed well in all work packages, and the first steps to the clinic have been made. Many interactions have also taken place between our NAIMIT consortium and other FP7 consortia dealing with type 1 diabetes, e.g. DIAPrepp (coordinator Prof. E. Bonifacio) and DIABIMMUNE (coordinator Prof. M. Knip). Also, active interactions have taken place with Beta Cell Therapy (coordinator Prof. D. Pipeleers), where the coordinator of NAIMIT also participated as a partner. In the dissemination arena, up to date, the internet page has attracted an important public. We have made professional contacts through the information on the web page. Also the number of scientific publications has increased from 11 in year one to 51 in year 5, with many collaborative publications. Importantly, the NAIMIT consortium not only performed high quality research, but also disseminated the results not only in peer-reviewed media, but also brought the findings to the greater public, in particular in Europe, but also in the rest of the world. Presentations on the website of NAIMIT (www.naimit.eu) support this mission.

1. Impact
NAIMIT has become a name with a content in the past 5.5 years. Researchers in the diabetes field, members of the press, but also EC itself has gotten to know this acronym as a synonym for a smoothly running consortium of high-level researchers in the field of type 1 diabetes who have jointly realised major steps in the path to a better understanding of the pathogenesis of type 1 diabetes and towards a cure for the disease. Impact has been important on researchers, on patients and their relatives, on society as a whole and interestingly also on industry.
NAIMIT has created a unique and tight collaborative network of researchers in the type 1 diabetes field in Europe, allowing these researchers to grow and through collaborations and exchanges to increase their impact and position in the field. Most researchers have in part thanks to NAIMIT succeeded in leveraging the funds obtained within NAIMIT and attract additional funding. Long-lasting collaborations have been forged, with joint PhD projects between partners, exchanges of personnel, common techniques and research tools and a major impact on the scientific output of all individual partners.

Major efforts were put not only on performing excellent research in the NAIMIT consortium, but also sharing the findings and ideas with the international research community, through our website (www.naimit.eu), but also through peer-reviewed publications in high-ranking journals and presentations at national and international conferences. Taking into account that the research performed within the frame of NAIMIT resulted in a total of 177 international peer reviewed publications, it is evident that this project had a large impact on researchers, within the consortium, but also in the whole international field.

The knowledge obtained over these 5 years has fundamentally changed our view on the pathogenesis of this devastating disease and will - or has already - influenced researchers in their way of thinking. Especially the concept that was set forward at the start of the NAIMIT project, namely that the beta-cell and the immune system are partners in crime in the pathogenesis of type 1 diabetes, has since the beginning of the project received a lot of support worldwide and at present, the central role of the beta-cell in its own destruction is accepted in the scientific community. The NAIMIT project and the partners of the consortium have contributed in a significant way to the elaboration and validation of this concept.

Impact on patients and their families: T1D affects more than 3 million individuals in Europe, with doubling of numbers expected in the coming 20 years. The majority of patients contract the disease at young age, with clinical presentation becoming more aggressive, leading to diagnosis in more children at younger ages. Although the numbers are smaller than for T2D, the condition has all the more impact since at present no cure exists and it affects individuals for the majority of their life. One of the most frustrating moments in a medical doctor's life is announcing the diagnosis of T1D to a patient and even more so to the parents of an affected child. At present, the only therapeutic option for the physician is to initiate insulin therapy and just be a bystander while the immune system further destroys the remaining beta-cells. Even with the best disease management, patients remain vulnerable to the devastating disease complications that damage in particular their eyes, kidneys, nerves and heart. While the importance of achieving normal blood glucose levels is clear, this therapy is particularly difficult. Patients are heavily involved in their own therapy and are very interested in finding a cure or a preventive intervention for their disease. In particular, patients worry about their children and relatives, as they are at increased risk. Patients thus are eager to learn about new research initiatives. Through our website, through lay publications, lay presentations done by every partner in their own countries, we have put a lot of effort in integrating the patients in our project. Their feedback is very useful. A special experience was the ‘Open door day’ of the EC where NAIMIT had an information stand explaining the research done within NAIMIT in very simple, but creative ways, with movies, hands-on experiences and direct contact with researchers.

The achievements obtained within the NAIMIT project, with phase I studies that are ready and approved to be initiated, i.e. for tolerogenic dendritic cell therapy; or are in the negotiating stages for final approval, i.e. for multipeptide therapies and therapies with Lactococcus lactis, may fundamentally change the way in which clinical interventions in T1D patients are performed in the near future. In addition, the findings obtained on the role of gene polymorphisms by pharmacogenetics, has opened the way to individualized therapies.

A special impact of NAIMIT was on the collaboration between the EC and JDRF. Shortly after the start of
NAIMIT, the consortium and project already attracted the attention of the JDRF, a charity organisation of parents of children with type 1 diabetes, who are leaders in sponsoring and directing research on type 1 diabetes worldwide. JDRF contacted the NAIMIT coordination team immediately after the start, and has gotten into contact with the EC via the NAIMIT consortium. Later on, this contact led to the IMI2 call on ‘Novel interventions in type 1 diabetes’, where JDRF participates on the side of the EFPIA partners.

Impact on society as a whole: On top of the burden T1D places on patients and their families, diabetes is an expensive disease. Both the daily treatment (including insulin analogues and intensive patient education), but especially the diabetic complications are a major financial burden. The cost of treating one patient over a 25 year period is in the range of 100,000 to 200,000 €. Next to these direct costs, indirect costs and emotional burden need to be taken into account. Scepticism exists on the relevance of further research efforts on finding a cure for T1D, nurtured by the failing of recent intervention trials and the impression that new drugs have solved all problems. Through the findings and results achieved within NAIMIT, we hope to fundamentally change the way clinical interventions will be performed in T1D patients in the near future, thereby maximizing the impact on society as a whole. We took our role as ‘role models of research’ very serious and have put a lot of focus in sharing our results in an understandable manner with non-scientists. In order for society to recognise the importance of EC-sponsored research, they need to be informed of the progress. Thus, we have established an accessible website, with movies and understandable explanations of the research. Also participating in dissemination activities in our local countries allowing non-researchers to have insight in what NAIMIT researchers were doing has contributed to the impact of NAIMIT on society.

Another effect of NAIMIT has been to put type 1 diabetes on the map with industry. Indeed, this rare disease does not attract major funding for new therapies, but in part thanks to the seriousness of the research within NAIMIT, industry has regained interest in the field and has even triggered the EFPIA-inspired IMI2 call on ‘Novel therapies in type 1 diabetes’.

2. Dissemination activities
The project website (www.naimit.eu) is available already since the start of the NAIMIT project (1st of November 2009) and has the logo of the European Commission, FP7 and NAIMIT. It contains an open section, aimed at researchers, patients and all interested members of society, with a summary of the project including background information and the aim of the FP7 project, information on the participants, NAIMIT open scientific meetings, NAIMIT meetings aimed at the general public, the NAIMIT publications, which are updated on a regular basis, as well as accessible movies with researchers explaining their work. Moreover, a password protected section for NAIMIT researchers, holds common protocols and techniques used by partners as well as postings on future meetings.

The NAIMIT website is monitored intensively, as it is linked to Google-Analytics. This makes it possible to give internal feedback to beneficiaries on where and when in the world researchers are accessing the website. Based on this feedback it is clear that the number of visitors is increasing (from 881 in year 1 to 1035 in year 2; to 1458 in year 3 and to 1317 in year 4, and to 2322 in year 5, see figure 2). Also, we believe the website is the ideal format to disseminate and promote the work performed within NAIMIT and we have therefore put a lot of content on the website, to increase the dissemination aspect of the NAIMIT project. This includes presentations for lay people and small videos, also published via Youtube (https://www.youtube.com/watch?v=8zG7uyX1yHQ and https://www.youtube.com/watch?v=2aMc1x1ikes) explaining the goals and results of the NAIMIT project.

Another way of disseminating the results obtained within NAIMIT was through publishing peer reviewed articles in international journals. The total number of publication shows a steady increase from year 1 (11
Particularly striking is the increase in the number of collaborative publications (2 in year 1 to 22 in year 5) with some publications having contributions from even 3 or 4 beneficiaries (Fig 3). This is a clear reflection of the actual collaborative activities that have taken place within the consortium. Other dissemination activities include publication of articles in the popular press, for a broader, non-scientific audience. As such, amongst many others, we have for instance written an article entitled ‘NAIMIT Natural Immunomodulation for intervention in Type 1 Diabetes’ or ‘Hoe kunnen we met natuurlijke afweermodulatie type 1 diabetes voorkomen? In het ‘Vlaams tijdschrift voor Diabetologie’ (see table 3). Another prominent way of disseminating the results was through invited oral presentations, on the ‘NAIMIT findings’, for which the coordinator has been invited at several occasions in different international fora (e.g. ISPAD (Pediatric Diabetes Society, IDS, EC).

NAIMIT has also received a lot of attention by the lay press. Different press releases have been launched, at crucial occasions during the project. For instance with the publishing of the preclinical results with the Lactococcus lactus strains obtained in the frame of WP4, published in J Clin Invest. During year 4 and 5 of NAIMIT even more efforts have been undertaken to disseminate results of NAMIT to a non-scientific audience. In this regard, the NAMIT project was selected to present its results during the EU Open Doors event, on the 4th of May 2013 (Fig 4). Also at KU Leuven, during the Opendoor day of ‘Day of Science’ the NAMIT project was selected and an event was organised to present its findings to patients and their families. Similar activities have taken place in The Netherlands, with for instance an ‘Infoday of the National Diabetes Center’ in Rotterdam on the 5th of Nov 2013. Moreover, special efforts have been made to explain the NAIMIT project and its results, by providing extra info on the NAMIT website, including 2 videos in which the different WPs are explained. These videos are also available on youtube and have already attracted a broad audience.

3. Exploitation

Most of the research performed within NAIMIT was preclinical, preparing for clinic. As such, the findings and preclinical research performed within the frame of WP1 on tolerogenic dendritic cells were essential for preparing and setting up a clinical trial that is currently being executed in LUMC. Also the knowledge gained in relation to restoring the T cell balance (WP2) using peptide immunotherapy was used to generate funding for a Phase I first-in-man study, which will start Q2 2015. The development of a peptide drug candidate has led to a formal license agreement between one of the academic partners (KCL) and UCB Pharma to continue this approach. Also for the SMEs, the results obtained within NAIMIT, increased the visibility and exploitability. The interesting preclinical data obtained with the L. lactis delivery platform for instance, positively contributed to the very recent acquisition of ActoGeniX by Intrexon (www.dna.com). This acquisition opens opportunities to pursue clinical development of the ActoBiotics™ therapy for Type 1 Diabetes. The second SME that was part of the NAIMIT consortium, Immunocore, has undergone a significant period of expansion following during this 4th year of NAIMIT. This has resulted in a boost in the last year of NAIMIT, with new human resources and financial strength to significantly expand its efforts with the establishment of a 10 person autoimmune effector group in the first 6 months of 2014.

List of Websites:
Website: www.naimit.eu

Contact details: