



REDDSTAR Project FINAL REPORT

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1. Final publishable summary report

1.1 Executive summary

Millions of patients with diabetes mellitus in the EU use prescription drugs to control their blood glucose levels. Poor control of blood glucose levels can lead to a number of complications, including: nephropathy (kidney disease), retinopathy (retinal disease), cardiomyopathy (heart disease), neuropathy (peripheral nerve dysfunction), impaired bone repair and wound ulceration. At present, there are few therapeutic options available to control diabetic complications.

REDDSTAR (Repair of Diabetic Damage by Stromal Cell Administration) has been focused on examining whether Mesenchymal Stromal Cells (MSC, from adult bone marrow) can safely control glycaemia and alleviate damage caused by six different diabetic complications.

The REDDSTAR project began on 1 November 2012 and finished on 31 October 2016. As part of our work, we successfully evaluated the effects of 3 MSC cell types in different models of diabetes. The team generated exciting pre-clinical results, demonstrating the positive effects of a novel MSC cell type (ORBCEL-M) on blood glucose, kidney disease, neuropathy and wound healing. In addition, our work has demonstrated the immunomodulatory effects of ORBCEL-M, in a pre-clinical model of diabetic cardiomyopathy. We have also seen its beneficial effect in ischaemic and diabetic retinopathy.

Based on our positive pre-clinical results, an independent panel at the Steno Diabetes Centre selected treatment of diabetic ulcers with ORBCEL-M for further exploration in a Phase 1b clinical trial. The Danish Medicines Agency (DKMA) and Danish National Committee on Health Research Ethics (NVK) recently approved the REDDSTAR Clinical trial authorisation application (CTA) to conduct this study.

Non-healing, neuro-ischaemic diabetic foot ulcers (DFU) are very common and present a significant burden on an individual's health and on healthcare systems. Despite current treatment strategies, there is a high prevalence of non-healing ulcers and amputation. Foot ulcers can affect 12% to 25% of people with diabetes mellitus throughout their lives. Right now there are more than 5 million patients in the US and EU suffering with non-healing DFU. REDDSTAR pre-clinical studies have highlighted the potential for a novel therapeutic product for people suffering with DFU, delivering improved wound healing and reduced healing time. The team is now planning for the conduct of the Phase 1b trial arising from REDDSTAR, to investigate the treatment of diabetic ulcers with ORBCEL-M. It is anticipated that the trial will commence in early 2017. The partners are committed to taking this next essential step towards novel clinical treatments for diabetic complications, with potential benefits for millions of patients worldwide. To this end the positive diabetic nephropathy data from REDDSTAR has already led to further research in H2020 project NEPHSTROM and a second planned clinical trial, this time in patients with diabetic kidney disease.

Within REDDSTAR we have also developed and validated a novel benchtop cell sorter, the MACSQuant® Tyto™, a high-speed, 10-parameter microchip-based cell sorter in a fully closed cartridge system, which offers rapid, multicolour flow sorting with high purity and ease of use. REDDSTAR was the first real world implementation of this novel technology which has far reaching value for cell purification in clinical and research use generally and cell therapy specifically. Cell purification is an important capability in therapeutic cell characterization, enhanced potency, minimization of confounding effects and reliable clinical performance. Alternative purification technologies have limitations with regard to sample/environment isolation, process control and utility in expertise-limited manufacturing environments. The MACSQuant® Tyto™ mitigates these limitations, thus broadly enabling progress in cell therapy development, and is now commercially available with early units being installed globally.

In the final analysis, we believe that REDDSTAR has generated exciting and novel results, with prospects far beyond the life of the project. Our findings have the potential to significantly impact the management and treatment of diabetes in the future, with benefits for patients, clinicians, researchers, pharmaceutical companies, health systems and for the general public.

1.2 A summary description of project context and objectives

The REDDSTAR project has focused on developing and testing stromal cell therapies to treat Diabetes Mellitus (DM). The key objective of the project has been to investigate the control of blood glucose by stromal cells, while also addressing a range of diabetic complications.

Background

DM is currently considered to be an epidemic global health problem. DM is a heterogeneous group of metabolic disorders characterized by hyperglycaemia (high blood glucose) with impaired metabolism of carbohydrate, fat, and proteins as a result of defects in insulin secretion, insulin action, or both. Both type 1 (T1DM) and type 2 (T2DM) cause blood glucose levels to increase above normal and current treatment strategies frequently result in suboptimal glycemic control, and may cause life-threatening hypoglycemic (low blood glucose) reactions. Over time, poorly controlled diabetes can lead to glucose toxicity, inflammation and a variety of serious secondary tissue complications, including heart disease, ulcerating wounds, blindness, amputations, kidney disease, and nerve damage. In fact, DM is the leading cause of blindness, end-stage renal disease, and a variety of debilitating neuropathies in the western world.

T2DM is a public health concern and projections of its future effects are alarming. According to the International Diabetes Federation, the worldwide prevalence of diabetes in 2015 is 415 million and is set to raise to 642 million by 2040 (International Diabetes Atlas 7th edition 2015; www.diabetesatlas.org/). The emerging pandemic is driven by the combined effects of aging population, increased urbanisation, changes in lifestyle with an increased caloric and dietary fat intake, rising levels of obesity and inactivity.

Millions of patients with DM in the EU are using prescription drugs to control their blood glucose levels. While insulin is the primary method of controlling diabetes by regulating blood glucose levels, insulin does not reverse or prevent disease progression or tissue damage. Improved glycemic control has been shown to slow the rate of progression of complications, but at the expense of hypoglycaemic reactions. There is no available therapy that will improve glycemic control and simultaneously treat the underlying diabetic complication(s). These distinct disorders have few effective medicines and present challenging management issues for clinicians.

The REDDSTAR Key Objective

Mesenchymal Stromal Cells (MSC) or Stromal Stem Cells (SSC) are a mixed population of plastic-adherent (PA) cells isolated from adult bone marrow. PA-MSC secrete potent immunosuppressive and angiogenic proteins (which stimulate new blood vessel formation). Accumulating evidence has shown that MSCs secrete potent immunomodulatory and angiogenic factors that may provide benefits in a variety of disease conditions and within REDDSTAR we examined their effects in pre-clinical models of diabetes.

The aim of REDDSTAR has been to comprehensively examine if MSC can safely control glycaemia and alleviate damage caused by six diabetic complications namely: nephropathy (kidney disease), retinopathy (retinal disease), cardiomyopathy (heart disease), neuropathy (peripheral nerve dysfunction), impaired bone repair and wound ulceration.

Prior to REDDSTAR, partner Orbsen Therapeutics identified a novel antibody that binds the surface proteoglycan Syndecan-2 (known as CD362) that can be used to prospectively isolate CD362⁺ SSC from human bone marrow with enhanced purity ratios. This new MSC platform technology (ORBCEL-M), is a radical improvement in terms of cell purity and compliance with upcoming clinical regulations REDDSTAR was split into several phases. Phase 1 of the project investigated the safety and efficacy of novel CD362⁺ MSC (ORBCEL-M), CD362⁻ MSC, and PA-MSC and their ability to simultaneously control hyperglycaemia and ameliorate diabetic complications. The second phase of the project involved examination of the mechanism of action (MOA) of how MSC improve diabetic complications. In the third and final phase of the project REDDSTAR partners continued with MOA studies but also submitted a clinical trial application to the Danish Medicines Agency (DKMA) and National Committee on Health Research Ethics (NVK) to undertake a clinical trial of the topical administration of ORBCEL-M to patients with non-healing diabetic ulcers (the complication that yielded the best results in phase 1 of the project). REDDSTAR partner OWL Biomedical/Miltenyi

Biotec also developed the world's first bench-top GMP-compliant cell sorter - the MACSQuant Tyto to produce CD362+MSC (ORBCEL-M) for human clinical safety trials that will meet future therapeutic regulatory requirements.

REDDSTAR has been unique in its approach, bringing together experts in six different diabetic complications to comprehensively study the response of all six complications to a single cell therapy, in five different models of diabetes, across three different species.

The overall objective of REDDSTAR has been to develop an MSC therapeutic that will comply with future regulatory requirements and that can be administered together with a patient's existing medications to simultaneously control hyperglycaemia and ameliorate a range of complications in diabetic patients. In order to allow us achieve our main objective, the following specific aims were set at the outset of the project:

1. To compare the safety and efficacy of novel CD362+ and CD362⁻ SSC to PA-MSC and their ability to simultaneously control hyperglycaemia and ameliorate diabetic complications.
2. To elucidate the mechanism of action of MSC in glycaemic control and the amelioration of each complication.
3. To ensure EU ATMP compliance and prepare an application for a Phase1b Clinical Trial of CD362 therapy of a chosen complication.
4. To carry out a Phase1b Clinical Trial of CD362 therapy of a chosen complication.
5. To maximise the long term research and collaboration value of REDDSTAR.

REDDSTAR Additional Objectives

In addition to our main objective, the REDDSTAR work plan also involved a number of ancillary objectives.

As part of the REDDSTAR project, the team also set out to meet the clinical need for a sterile, high-speed, high volume, high-viability, easy to use, and disposable system for cell sorting, an essential requirement for cell-based therapies to become clinically viable. The intention was to develop the world's first cGMP-compliant FACS nanosorter, (the Tyto) specifically for the isolation of therapeutic stromal cells. Cell purification is an important capability in therapeutic cell characterization, enhanced potency, minimization of confounding effects and reliable clinical performance. Alternative purification technologies have limitations with regard to sample/environment isolation, process control and utility in expertise-limited manufacturing environments. Accordingly, successful development of the Tyto would radically change the way cells are sorted for GMP manufacturing and therapy development.

In REDDSTAR we also wanted to facilitate the sharing, management and backup of the research data emanating from the pre-clinical work undertaken. In order to analyse and cross correlate the data for several different complications (the markers, measurements, and factors which are recorded by the researchers), the data needed to be accessible and harmonised across the consortium. In order to meet this need the team set out to develop a platform that enabled multi-national researchers, clinicians, and analysts to share research data securely online.

In addition to the above, the REDDSTAR team planned to maximise the value of the project (i) by communicating the project concept, progress and results to a range of audiences (including life science researchers, clinicians, drug development companies, the general public, the media, advocacy and patient representative groups, other relevant EU projects and young people) and (ii) by focusing on the future exploitation and commercialisation of REDDSTAR project results.

Conclusion

In summary then, the main aims of REDDSTAR have been to significantly impact the management and treatment of diabetes, with relevance for clinicians, researchers in diabetes and regenerative medicine, drug/pharmaceutical companies, patients and for the general public. Our aim has been to improve the treatments available for diabetic patients, and to enhance their health and quality of life. Ultimately it is hoped that such improvements can lead to a reduction in the public health costs associated with diabetes and related complications.

1.3 A description of the main S&T results/foregrounds

1.3.1 Introduction

In REDDSTAR a multi-disciplinary team has come together to explore the application of stromal cell therapies to treat Diabetes Mellitus (DM) and related complications. The key aim of the project has been to comprehensively examine if MSC can safely control glycaemia and alleviate damage caused by six diabetic complications namely: nephropathy (kidney disease), retinopathy (retinal disease), cardiomyopathy (heart disease), neuropathy (peripheral nerve dysfunction), impaired bone repair and wound ulceration. Our ultimate goal has been to significantly impact the management and treatment of diabetes, with relevance for clinicians, researchers in diabetes and regenerative medicine, drug/pharmaceutical companies, patients and for the general public.

Phase 1 of the project investigated the safety and efficacy of novel CD362⁺ MSC (ORBCEL-M), CD362⁻ MSC, and PA-MSC and their ability to simultaneously control hyperglycaemia and ameliorate diabetic complications. The second phase of the project involved examination of the mechanism of action (MOA) of how MSC improve diabetic complications. In the third and final phase of the project REDDSTAR partners continued with MOA studies but also submitted a clinical trial application to the Danish Medicines Agency (DKMA) and National Committee on Health Research Ethics (NVK) to undertake a clinical trial of the topical administration of ORBCEL-M to patients with non-healing diabetic ulcers (the complication that yielded the best results in phase 1 of the project). REDDSTAR partner OWL Biomedical/Miltenyi Biotec also developed the world's first bench-top GMP-compliant cell sorter - the MACSQuant Tyto to produce CD362⁺MSC (ORBCEL-M) for human clinical safety trials that will meet future therapeutic regulatory requirements.

The main research undertaken and results achieved are described below under the following headings:

- WP1 SSC Platform
- WP2 Diabetic Neuropathy
- WP3 Diabetic Retinopathy
- WP4 Diabetic Cardiomyopathy
- WP5 Diabetic Nephropathy
- WP6 Diabetic Ulcers
- WP7 Diabetic Bone Fractures
- WP8 Data Management
- WP9 GMP Cell Production
- WP10 Clinical Trial

1.3.2 WP1 SSC Platform

1.3.2.1 Introduction

Human tissue-derived MSC comprise a mixed population of fibroblastic cells that can be isolated from bone marrow by adherence to tissue culture plastic and formation of colony forming unit-fibroblasts (CFU-F). A major hurdle in the development of MSC therapies in the past has been the absence of a single characteristic or marker with which to define MSC. ORB however, have identified a novel MSC marker CD362 (Syndecan 2), a heparan sulfate proteoglycan, a stromal cell protein that labels MSC isolated from human, murine, equine, rat and rabbit marrow allowing for the prospective isolation of a defined MSC population.

The purpose of WP1 was to supply the pre-clinical partners (WP2, WP3, WP4, WP5, WP6 and WP7) with sufficient CD362 selected (CD362⁺ and CD362⁻) and unselected MSC to conduct preclinical experiments. In addition, ORB aimed to explore the efficacy, toxicity and biodistribution of human stromal cells (selected and unselected) in preclinical

models of Type 1 diabetes. Finally, as ORBCEL-M (CD362-selected MSC) was selected by the Steno team to progress as a clinical therapy for non-healing neuroischemic diabetic foot ulcers, our work in P3 of REDDSTAR was focused on developing a GMP ready formulation of ORBCEL-M in a collagen scaffold for use in the REDDSTAR BMW trial.

1.3.2.2 Key Work Undertaken and Results Achieved

Supply of MSCs for pre-clinical studies (CD362+, CD362-, PA-MSC)

WP1 provided MSCs for all pre-clinical studies in the REDDSTAR project. ORB established GLP SOPs for preparation and FACS isolation of CD362⁺ MSC from human marrow and umbilical cord tissues. Tech transfer of the marrow-derived CD362 SOP was completed by ORB meeting with OWL and LUMC partners to demonstrate the SOPs on human marrow samples. This tech transfer was essential to enable further development of the MACSQuant Tyto sorting protocols in WP9.

ORB established protocols for (i) MSC in vitro expansion in culture, (ii) MSC cryopreservation, (iii) MSC shipping to WP partners, (iv) thawing and culture of cryopreserved MSC at partner site and (v) adoptive transfer of MSC to preclinical models by intravenous injection. Using the SOPs, ORB banked MSC from human donors and supplied MSC for testing in different models of diabetes and vascular complications. This work continued throughout the project as WP1 supplied essential raw material for all the REDDSTAR preclinical WPs.

MSCs: Isolation and Testing Toxicology, Efficacy and Biodistribution

In WP1 we also sought to further define the marrow-derived CD362 MSC therapy, ORBCEL-M. ORB isolated CD362⁺ MSC from human umbilical cord, which may represent a superior source of cells. CD362⁺ MSC were also isolated from human adipose tissue. To better understand the biology of the CD362 protein and its relation to stromal cell biology, ORB demonstrated that CD45⁻ CD362⁺ MSC can be isolated from murine marrow, adipose tissue, skeletal muscle, lymph nodes, spleen and thymus. CD362⁺ MSC were also isolated from equine marrow, rat marrow and rabbit marrow.

Studies undertaken regarding efficacy, toxicology and biodistribution of MSCs included:

- *Study to assess the extent to which human CD362⁺, CD362⁻ and PA-MSC could safely reverse streptozotocin (STZ)-induced hyperglycemia in STZ-treated NOD/SCID mice:* An STZ model of induced diabetes in NOD scid mice was established at ORB using 40mg/kg of STZ over five consecutive days and was used to test the effect of the three MSC cell types on streptozotocin (STZ)-induced hyperglycemia. Through these studies we found that although MSC may prevent the onset of diabetes (initial studies) in this model they do not reverse already established diabetes.
- An additional study investigated the effects of cell administration to mice with spontaneous diabetes. A colony of NOD mice was established at ORB. The incidence of diabetes was 78% in females and 26% in males by 30 weeks in line with incidence rates in SPF-free facilities. A high dose of hPA-MSC delayed the onset of T1DM in this population by 1 week.
- *Study to determine the distribution and persistence of hMSC DNA at 2 weeks when administered by the IV route to genetically immunodeficient mice:* 23 diabetic NOD scid mice were injected intravenously (IV) with 1×10^6 human MSCs (hMSCs) of bone marrow (BM) or umbilical cord (UC) origin or vehicle, on day 17 and again on day 24 following STZ administration. 2 weeks following cell administration analysis of the biodistribution and persistence of hMSCs was assessed by qPCR analysis using a biodistribution SOP developed in REDDSTAR. Eleven organs were subjected to this assay including bladder, femur, heart, kidney, large intestine, liver, lung, pancreas, small intestine, spleen and stomach. Overall human DNA was detected in the lungs of animals treated with human umbilical cord-derived MSCs (hUC) at 2 weeks. but in animals treated with hBM-derived cells results were not statistically different from the saline treated animals, indicating lack of detection within the limits of our biodistribution SOP. hDNA was not detected in any other tissue within the limits of this PCR based biodistribution assay. The results found here are in line with what was found with other REDDSTAR partners after I.V. delivery of stromal cells, and they are also in line with the

recent literature which has shown that human MSC's applied I.V. to pre-clinical animal models accumulate in the lungs and are rapidly cleared from the circulation within 3 days.

- *Single dose toxicity study of REDDSTAR ORBCEL-M cell product in genetically immunodeficient mice:* Orbsen contracted an outsourced GLP company to perform a single dose toxicity study of REDDSTAR ORBCEL-M cell product in genetically immunodeficient mice. ORBCEL-M was administered intravenously at a dose of 1×10^6 cells to Nu/Nu mice once on Day 0. Endpoint of the study was Day 90. Endpoint measurements included safety (general health observations, body weights, appetite, haematology, coagulation, clinical chemistry, and histology). No abnormal ORBCEL-M – related signs were noted throughout the study. ORBCEL-M cells did not affect weekly body weight or appetite. There were no meaningful clinical findings for haematology, coagulation and clinical chemistry. No abnormalities related to ORBCEL-M were observed macroscopically at necropsy or microscopically via histological evaluation. Early deaths were not related to the test articles. In our biodistribution analysis we were unable to detect the presence of human genomic DNA in the sampled tissues from animals administered Test Article 1 ORBCEL-M™ (Group 3) 90 days after cell administration.

The above studies have given rise to important new knowledge about MSCs, their efficacy, safety and biodistribution. Through evaluations carried out by pre-clinical partners in REDDSTAR, ORB further ratified the suitability of their novel MSC marker (CD362 (Syndecan-2)) and successfully achieved a patented product (ORBCEL).

Documentation for REDDSTAR Clinical Trial

A major part of our work in WP1 has also been related to the preparation of an Investigational Medicinal Product Dossier (IMPD) and Investigators Brochure (IB) as part of the REDDSTAR Clinical Trial Application to the Danish Medicines Agency. Partners (ORB, LUMC, NUIG, Steno) worked closely together for the development of the final REDDSTAR clinical production process and final product formulation (REDDSTAR ORBCEL-M) to deliver ORBCEL-M topically to non-healing ulcers in Diabetic patients. As part of this work, a strategy was developed to manufacture the trial drug formulated in a buffer with appropriate cell survival capacities and then mix on-site with a collagen solution.

Before production for the REDDSTAR clinical trial, the formulation and packaging were validated with 3 validation runs. The procedures were performed with the materials / under the circumstances (disposables / locations) that are used/maintained during ATMP production. All measurements from the validation runs were well within specifications. Detailed procedures were developed for generation of the final drug product covering e.g. packaging, transport, checks on receipt, recording of data, preparation for administration and mixing with collagen solution. Several optimisation experiments were performed to assess the mixing process and develop the mixing SOP. Cell viability was confirmed by active growth post mixing. The result of the procedure is a single therapeutic dose of cells in a collagen solution that can be applied at a specified dosage to the wound area of a DM patient with a non-healing leg ulcer.

A final version of the REDDSTAR IMPD and IB (containing the final REDDSTAR clinical production process and final product formulation) was prepared and submitted to the Danish regulator (DKMA) in June 3rd 2016. These documents were approved by the DKMA and NVK (Ethics) in August 2016, paving the way for the REDDSTAR clinical trial.

1.3.2.3 Conclusion

Within WP1 the team has succeeded in:

- Isolating, expanding and cryopreserving CD362⁺/CD362⁻ SSC and PA-MSC from the bone marrow of human donors under GLP conditions.
- Supplying vials of high quality, cryopreserved human stromal cells to REDDSTAR partners for pre-clinical testing in WP2-WP7
- Providing GLP-compliant REDDSTAR Standard Operating Procedures (SOPs) and Batch Manufacturing Records (BMR) to REDDSTAR partners to define, govern and record the thawing and expansion of cryopreserved CD362⁺/CD362⁻ SSC and PA-MSC in each WP laboratory.

- Evaluating toxicology, anti-glycemic efficacy and biodistribution of human CD362-MSC administered to immunocompromised NOD scid and diabetic NOD mice.
- Preparing the REDDSTAR ORBCEL-M Investigation Medicinal Product Dossier (IMPD) and Investigators Brochure (IB) for submission to DMA as part of Clinical Trial Application and developing the final ORBCEL-M/Collagen transportation and mixing protocols for the Phase 1b clinical trial – which was approved for use in patients by DKMA on August 3rd 2016.

1.3.3 WP2 Diabetic Neuropathy

1.3.3.1 Introduction

Diabetic neuropathy (DN) is one of the most common and devastating diabetes complications, with several possible clinical manifestations [1]. Patients with DN may develop painful symptoms and/or present abnormal pain responses. These patients very often undergo episodes of pain described as deep aching, sharp, electric shock-like, prickling or stabbing, burning. The presence of allodynia (painful sensations to innocuous stimuli) and hyperalgesia (increased sensitivity to painful sensations) is also very frequent. Such conditions have enormous physical and psychological impacts, affecting the patient's ability to properly maintain work, mood, and quality of life. Moreover, current treatment strategies are still very limited, and most of them present undesired side effects [2].

The main objectives for WP2 were to:

1. Compare the **efficacy** of intravenous and intrathecal administrations of CD362⁺, CD362⁻, and plastic adherent (PA) mesenchymal stem cells (MSCs) in the reversal of peripheral and central features of experimental **diabetic neuropathy**.
2. Ascertain the **neurobiological mechanisms** underlying the neuroprotective effects of MSCs at pain control centres of the brain, hypothesizing an IGF-1-based mechanism of action.

1.3.3.2 Key Work Undertaken and Results Achieved

Vials of 1×10^6 cryopreserved human bone marrow plastic adherent (PA), CD362⁺, and CD362⁻ mesenchymal stem cells (MSCs) (at Passage 2/3) were provided by partner ORB. At UPORTO, MSCs were re-seeded at 1×10^6 MSCs per T-175 flask, expanded, and aliquots of 2.5×10^6 MSCs were cryopreserved in Freezing Medium at Passage 3/4 in liquid nitrogen until needed. To prepare cells for transplantation, MSCs were re-seeded at 2.5×10^6 MSCs per T-175 flask and allowed to grow to near confluency.

Animal experiments were performed using a well-established rat model of T1DM that develops DN-associated altered pain responses. Briefly, T1DM was induced in male Wistar rats by an intraperitoneal injection of streptozotocin (STZ) (60 mg/kg) dissolved in 0.1 M citrate buffer pH= 4.5, after a 6-hour fast. Sham control animals received an equal volume of vehicle solution (0.1 M citrate buffer, pH= 4.5). Three days post-STZ injection, blood glucose levels were quantified in tail vein blood samples STZ-injected rats with glycemic values higher than 300 mg/dL were considered diabetic and included in the study.

We started the in vivo studies by undertaking a dose study testing three systemically administered doses of CD362⁺ MSCs: 1×10^6 , 2×10^6 , and 4×10^6 . We were able to show that the intermediate dose of 2×10^6 CD362⁺ MSCs is the most effective in preventing the development of behavioural signs of DN-associated altered nociception in our rat model of T1DM. Having ascertained 2×10^6 as the most efficacious CD362⁺ MSC dose, we proceeded towards comparing the efficacy of the CD362⁺ MSC population with that of the PA and CD362⁻ MSC populations. The results of this head-to-head study provided evidence suggesting CD362⁺ MSCs as the most effective MSC population in preventing the development of behavioural signs of DN-associated altered pain responses. Moreover, we showed that none of the different CD362⁺ MSC doses, nor the different MSC populations tested had a detectable effect on body weight, glycemia, or HbA1c levels, suggesting that mechanisms besides glycemic alterations should account for behavioural signs of diabetic neuropathy in diabetic rats. Further to the behavioural analyses, the efficacy of the

different MSC populations in preventing the development of structural signs associated with the development of DN, specifically the loss of intraepidermal nerve fibers, was evaluated. However, since STZ-diabetic rats did not exhibit decreased intraepidermal nerve fiber density (IENFD)—examined in hindpaw plantar skin sections—as compared to control animals, the use of IENFD as a means to assess the efficacy of MSCs administration was discontinued.

Following the dose and efficacy studies, we started investigating possible neurobiological mechanisms underlying the protective effects of the CD362⁺ MSCs in our rat model of T1DM with DN-associated altered pain responses. We hypothesised that CD362⁺ MSCs may exert neuroprotective actions in DN-associated altered nociception through the paracrine modulation of the levels of inflammatory and/or angiogenic mediators, as well as of the levels of trophic factors, in peripheral and/or central nervous system tissues involved in the pain pathway. Hence, sciatic nerve, spinal cord, and prefrontal cortex samples from controls, STZ-diabetic, and STZ-diabetic animals administered 2×10^6 CD362⁺ or CD362⁻ MSCs were used for determining the levels of inflammatory and angiogenic mediators, and of trophic factors through quantitative Western blotting analyses. Furthermore, using this methodology, we evaluated other possible candidate mediators of the neuroprotective actions of CD362⁺ MSCs such as makers of synaptic integrity, a myelin marker, and cytoskeleton proteins. The data we obtained supports the existence of a peripheral effect, suggesting modulation of sciatic nerve inflammation as one of the possible underlying mechanisms of the protective actions of CD362⁺ MSCs in our rat model of T1DM.

Given the observed protective effects of intravenously delivered CD362⁺ MSCs in not only our animal model of DN but also in animal models of other diabetes complications, namely diabetic kidney disease and diabetic retinopathy, in the absence of glycemia normalisation, a common mechanism of action relying on a systemic effect emerges as being a plausible hypothesis. In this context, we decided to evaluate the paracrine effects over time of systemically administered CD362⁺ to STZ-diabetic rats through the analysis of a panel of cytokines/chemokines/trophic factors/adhesion molecules circulating in the blood using state-of-the-heart Luminex Multiplex Array analyses. Most importantly, the search for CD362⁺ effector molecules in the blood has the potential not only to provide clues to their mechanism of action, but also to provide valuable biomarkers for monitoring CD362⁺ MSCs clinical efficacy. Our data suggest that CD362⁺ MSCs may, indeed, exert systemic protective actions in our rat model of T1DM through the maintenance of elevated levels of immune-protective molecules.

1.3.3.3 Conclusion

Painful DN is a devastating complication of diabetes for which current treatment strategies are still very ineffective.

Under REDDSTAR WP2, we were able to show that the dose of 2×10^6 CD362⁺ MSCs (when compared to 1×10^6 and 4×10^6 MSCs) is the most effective in preventing the development of behavioural signs of DN-associated altered nociception in our Wistar rat model of T1DM. Further, we provided evidence suggesting CD362⁺ MSCs (amongst PA and CD362⁻ MSCs) as the most effective MSC population in preventing the development of behavioural signs of DN-associated altered pain responses. Moreover, we showed that none of the different CD362⁺ MSC doses, nor the different MSC populations, tested had a detectable effect on body weight, glycemia, or HbA1c levels. As to the neurobiological mechanisms underlying the protective effects of the CD362⁺ MSCs, we showed the existence of a peripheral effect, suggesting modulation of sciatic nerve inflammation as one of such mechanisms. Furthermore, we showed that CD362⁺ MSCs may also exert systemic protective actions in our rat model of T1DM through the maintenance of elevated levels of immune-protective molecules. Importantly, these molecules may serve as valuable biomarkers for monitoring CD362⁺ MSCs efficacy.

1.3.3.4 References

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1.3.4 WP3 Diabetic Retinopathy

1.3.4.1 Introduction

Current therapies for diabetic retinopathy (DR) are mainly focused on end-stages of the disease and do not address the primary pathology. Cell-based therapy provides a promising strategy to address pericyte loss and microvascular insufficiency in early diabetic retinopathy. We have focused on the potential for CD362⁺ MSC (ORBEL-M) to regulate retinal vascular repair and vessel integrity in ischaemic retinopathy (IR) following direct delivery into the vitreous. We also sought to assess the potential benefit of this highly-defined population of human bone marrow-derived CD362⁺ MSC in a murine model of DR following both direct injection into the vitreous and following systemic delivery.

1.3.4.2 Key Work Undertaken and Results Achieved

We present two main bodies of work: Direct delivery of CD362⁺ MSC into the ischaemic retina using the Oxygen-Induced Retinopathy (OIR) model and systemic delivery of CD362⁺ cells into diabetic mice (Diabetic Retinopathy model).

Ischaemic Retinopathy:

C57/Bl6 mice pups (postnatal day 7, P7) were subjected to an experimental model of oxygen-induced retinopathy by exposure to high oxygen (75% oxygen, 5 days). At P13 mice received a 1µl intravitreal injection in one eye containing Qdot nanocrystal labelled CD362⁺, CD362⁻, plastic adherent MSCs (PA-MSCs) or CD362⁺ MSC-conditioned media (CM). Cell numbers delivered were low [1×10^3], medium [1×10^4] or high [1×10^5]. The contralateral eye was injected with vehicle (DMEM) as a control. Three days post-injection, retinal flatmounts were processed and stained with isolectin B4/streptavidin-AlexaFluor488, and imaged using confocal microscopy. Retinal vasculature was quantified using ImageJ software (avascular or neovascular area/total area, %). CD362⁺ MSCs localised to the murine retina in a perivascular manner. Intravitreal delivery of CD362⁺ MSCs showed a significantly reduced avascular area than control at medium (P=0.031, n=6) and high cell numbers (P=0.046, n=11). No difference was found for low dose (P=0.507, n=5). CD362⁻ MSCs also promoted revascularisation at medium cell dose only (P=0.038, n=9). PA-MSCs did not have a significant effect on avascular areas for all doses (low: P=0.739, n=4; medium: P=0.085, n=8; high: P=0.741, n=12). This increased revascularisation may be a result of some CD362 cells associating with host vasculature. No difference was found in neovascularisation areas (P>0.050) for all groups. When treated with CD362⁺MSC-CM, both avascular (P=0.006, n=9) and pre-retinal neovascular areas (P=0.019) were significantly reduced.

As a complementary in vitro study to assess the impact of CD362⁺ MSC interacting with the vasculature, we performed in vitro tubulogenesis under hypoxia (1% O₂) using two inter-related approaches: 1) A Matrigel assay using endothelial colony forming cells (ECFCs) (7×10^4) mixed with CD362⁺MSCs (1×10^4) were suspended in Matrigel which was spotted onto 24 well plates; 2) A double-layer Matrigel assay in which ECFCs were suspended in Matrigel and spotted onto 24 well plates followed by topping of another Matrigel layer containing CD362⁺ MSCs one day later. Tube-like structures were imaged using confocal microscopy or conducted for immunostaining assay.

The pericyte markers, NG2, PDGFR β , α -SMA were detected in both CD362⁺ MSCs and CD362⁻MSCs, in which significant increase of α -SMA expression cells were found in CD362⁺ MSCs compared to CD362⁻ MSCs (45.7% \pm 0.04 in CD362⁺ MSCs and 24.5% \pm 0.06 in CD362⁻ MSCs, p=0.0306, n=4). In the in vitro angiogenesis assay, regression of ECFC tubules occurred at 1 week while the presence of CD362⁺ MSCs stabilized the network with the cells taking on a perivascular location. CD362⁺ SSCs /ECFC co-culture maintained stabilized vascular networks until two weeks by showing significantly enhanced tube area (p=0.005, n=4) and branch points (p<0.0001, n=8) compared to ECFCs alone. In the double-layer Matrigel assay, CD362⁺ MSCs migrated into "pre-formed" ECFC networks and took up a perivascular position. Pericyte markers were expressed in those CD362⁺ MSCs resident alongside tubes.

Diabetic Retinopathy:

C57/Bl6 male mice (12 weeks old) were injected with streptozotocin (in citrate buffer, 5x single IP injections/day, 50 mg/kg) to induce diabetes (DB) while other animals received citrate buffer alone (non-diabetic controls, NDB).

Groups were kept for 6 months, blood glucose and body weight monitored monthly. At 6 months post-induction DB mice received a single dose of 100µl of 1×10^6 CD362⁺ SSCs (n=7) or CD362⁻ SSCs (n=6) in DMEM, delivered via intravenous (tail-vein) injection. Control groups of DB (n=5) and NDB (n=8) mice received PBS injections. Four weeks post-treatment, in vivo retinal function (scotopic electroretinogram, ERG; stimuli range 0.008-25cd.s.m⁻²) and thickness (OCT) were measured. Eyes were enucleated and processed for immunofluorescence staining on retinal flat-mounts and cryosections (isolectin B4/collagen IV; Brn3a/Iba-1), and imaged using confocal microscopy. Acellular capillaries, retinal ganglion (RGC) and microglial cell(MC) counts were performed. qPCR analysis of retinal tissue show no human DNA detected 3 days after treatment..

We found that retinal function in DB mice revealed a significant reduction in photoreceptor (a-wave, $P < 0.01$) and ON-bipolar cell responses (b-wave, $P = 0.01$) compared with NDB group. Mice treated with CD362⁻ MSCs showed an improved ON-bipolar cell response similar to NDB controls ($P > 0.05$). The retina was significantly thinner in DB mice treated with PBS and CD362⁻ MSC than NDB mice ($P < 0.0001$), however CD362⁺ MSC treatment maintained retinal thickness ($P > 0.05$). There were reduced Brn3a-positive RGCs in DB mice ($P = 0.04$ vs NDB), while MSC-treated DB groups showed significant retinal neuroprotection. Analyses of Iba-1 positive MCs showed that CD362⁺ treated mice had less MCs in the retina. Increased acellular capillary formation (hallmark of DR progression) occurred in DB mice ($P < 0.05$ vs NDB). MSC treatment prevented capillary loss, with CD362⁺ MSC ($P < 0.01$) being more effective than CD362⁻ MSC ($P < 0.05$).

1.3.4.3 Conclusion

We conclude that CD362⁺ MSCs (ORBCEL-M)_associate closely with the host vasculature and appear to act as pericyte progenitors by showing the capacity to associate with vascular tubes and stabilize the network under hypoxia conditions. In the OIR model, these cells have promise for cell-replacement therapy for retinal ischemic diseases by promoting revascularisation of the ischaemic retina via two routes: 1) secretion of complex paracrine factors and 2) differentiating into pericytes and promoting vascular stability. These findings suggest that human CD362⁺MSCs may have utility for cell therapy to address retinal ischaemia when delivered directly into the vitreous.

CD362⁺ MSC also have significant retinoprotective properties by protecting against progression of key neurovascular pathology observed in DR. Although few cells reach the retina via the systemic route, this delivery does have significant benefit although the precise mechanism is unclear at this point.

1.3.5 WP4 Diabetic Cardiomyopathy

1.3.5.1 Introduction

Cardiovascular complications are the main cause of morbidity and mortality in individuals with diabetes mellitus. Besides hypertension and coronary artery disease, chronic heart failure in diabetic patients is also caused by “diabetic cardiomyopathy”. Diabetic cardiomyopathy is a cardiac disorder, which takes place independent of hypertension or coronary artery disease, and is characterized mainly by interstitial inflammation, cardiomyocyte oxidative stress, interstitial and perivascular fibrosis, cardiomyocyte apoptosis, intramyocardial microangiopathy, and endothelial dysfunction (2, 3, 4). Several lines of evidence indicate that left ventricular (LV) diastolic dysfunction represents the earliest preclinical manifestation of diabetic cardiomyopathy and illustrate that it can progress to symptomatic heart failure (5, 6).

Mesenchymal stromal cells (MSC) are an attractive cell type for cell therapy given their immunomodulatory, anti-fibrotic, pro-angiogenic, endothelial-protective features, their ability to be used allogeneically, and their capacity to home to injured tissues after intravenous (i.v.) application. With respect to diabetes mellitus, MSC have been shown to exert anti-diabetic effects and to improve experimental diabetic cardiomyopathy (7, 8). The effect of MSC on the onset of diabetic cardiomyopathy and its pre-clinical manifestation, diastolic dysfunction, has not been unravelled so far. Inflammation, in particular, is an important characteristic of diabetic cardiomyopathy.

Systemic delivery of MSC (Figure 1 below) associated with non-cardiac-specific targeting (including among others the spleen and pancreas) might even have advantages over intramyocardial (i.m.), i.e. cardiac-targeted injection. This follows from 1) the link between inflammation and fibrosis (9); 2) the importance of the cardiopleuric axis in the induction of cardiac inflammation (10); 3) the immunomodulatory features of MSC including the release of tumor necrosis factor-inducible gene 6 protein (TSG-6) upon arrival in the lung following i.v. application (11), and 4) the anti-diabetic effects of MSC.

In this respect, the aims of WP4 / diabetic cardiomyopathy were to evaluate the effects of stromal cells (MSC) (CD362⁺, versus CD362⁻, and plastic adherent SSC (PA-SSC or wild-type (wt) MSC)) after i.v. and i.m. application in different experimental models of diabetes mellitus:

- 1) the streptozotocin (STZ)-induced diabetes mellitus model
- 2) the db/db mouse model

Since the immunomodulatory effects of MSC after i.v. application importantly contribute to their cardioprotective effects (11), the evaluation of the immunomodulatory effects of MSC was one of our major points of investigation.

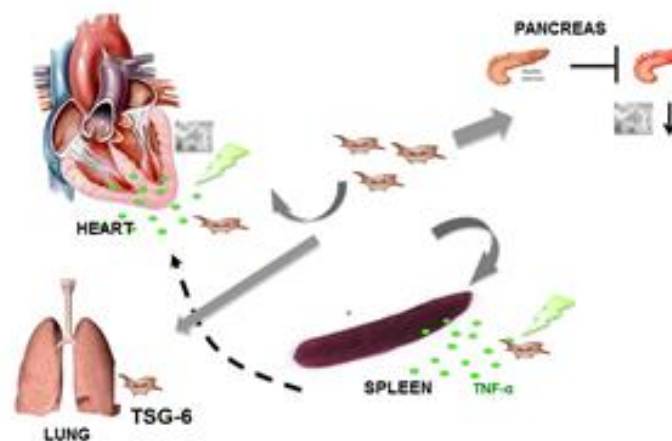


Figure 1 Hypothetical working mechanism how i.v. MSC application might reduce diabetic cardiomyopathy. 1) via direct cardioprotective effects, 2) via immunomodulatory effects, 3) via reducing blood glucose levels and 4) via release of tumor necrosis factor-inducible gene 6 protein (TSG-6) upon embolization in the lung.

1.3.5.2 Key Work Undertaken and Results Achieved

In vitro evaluation of the impact of an inflammatory environment, mimicked by TNF- α supplementation, on the TSG-6 mRNA expression of wt, CD362⁺, and CD362⁻ cells revealed that TSG-6 mRNA expression was extensively induced in all cells and that CD362⁻ cells expressed more TSG-6 than wt-MSC or CD362⁺ cells. TSG-6 protein levels in the supernatants were undetectable.

Comparison of i.v. application of wt, CD362⁻, and CD362⁺ MSC in db/db mice (C57BL/6 background, injection at 11w, followed by sacrifice at 15w) at an early stage of diabetic cardiomyopathy, with no prominent cardiac phenotype, demonstrated that the systemic immunomodulatory effect was the most pronounced after CD362⁺ cell application in terms of reduction in diabetes-induced proliferation of mononuclear cells, increase in regulatory CD4-IL10-expressing cells, and a decrease in TNF- α -expressing CD68 cells.

After intensive search for a more pronounced form of diabetic cardiomyopathy in db/db mice via performing a timeframe experiment in db/db mice with C57BL/6 background (Janvier) and via evaluation of db/db mice in the C57BLKS background (Taconic), the impact of i.v. application of wt, CD362⁻, and CD362⁺ MSC was evaluated in these db/db mice. We found that LV function improved after i.v. injection of wt-MSC and CD362⁻ cells, but not after

injection of CD362⁺ cells in db/db C57BLKS mice. In agreement with the LV function, the down-regulated phosphorylation state of the sarcomere protein titin N2B, which is besides collagen of high importance for LV function, was only induced after application of wt-MSC and CD362⁻.

For the i.m. cell application, an image-guided needle injection (IGNI) was set-up. Similar to i.v. administration, i.m. application of CD362⁺ cells in db/db mice did not improve LV function.

Comparison of i.v. application of wt, CD362⁻, and CD362⁺ MSC in STZ-induced diabetic mice revealed that CD362⁺ cells were superior over wt MSC and CD362⁻ cells with respect to immunomodulation in STZ mice. At this stage of STZ-induced diabetic cardiomyopathy associated with impaired diastolic dysfunction (potentially due to cardiomyocyte stiffness), but without pronounced cardiac inflammation and fibrosis, only an improvement in diastolic function was seen after wt and CD362⁻ MSC application, but not after CD362⁺ cell application.

With respect to i.m. application of wt, CD362⁻, and CD362⁺ MSC in STZ-induced diabetic mice, an improvement in LV function was observed by all cells.

1.3.5.3 Conclusion

I.v. and i.m. application of wt, CD362⁻, and CD362⁺ cells induced systemic immunomodulatory effects and did not decrease blood glucose levels in both STZ-induced diabetic and db/db mice. The superiority of CD362⁺ MSC in immunomodulation on the one hand and their ineffectiveness to improve LV function after i.v. application in STZ and db/db mice, suggests that systemic immunomodulation cannot be the main mechanism underlying the improvement in LV function following i.v. application of wt-MSC and CD362⁻ in both diabetes models. The observation that CD362⁺ MSC only improve LV function after i.m. application in STZ mice, indicates that the cardioprotective effects of those cells depend on the application route used as well as on the state of diabetes (STZ versus db/db).

1.3.6 WP5 Diabetic Nephropathy

1.3.6.1 Introduction

Mesenchymal stem cells are defined as multipotent, heterogeneous populations of cells that can differentiate into various cell types such as bone and cartilage cells etc. Mesenchymal stromal/stem cells (MSC) have become a potential therapeutic option for various inflammatory diseases. SSC as a therapeutic intervention have been reported in myocardial infarction [1], colitis [2], liver failure [3], kidney failure [4], Crohn's disease [5], central nervous system (CNS) trauma [6], and several autoimmune diseases [7–9].

Hence in the current project we aimed to study the efficacy of ORBCEL-M in the treatment of diabetic nephropathy using a uninephrectomized db/db mouse model with the following objectives.

Objectives of WP5:

1. To optimize the effective dose and timing of purified, characterized MSC on progressive DN.
2. To compare the protective efficacy of CD362⁺, CD362⁻ and PA-MSC in experimental DN.
3. To identify the distribution of GFP positive CD362⁺, CD362⁻ and PA-MSC in experimental DN.
4. To identify a urinary biomarker of MSC bioactivity in DN mice for potential use in human trials.
5. To evaluate effects of MSC therapy on kidneys of the disease models used in WP2, WP3, WP4, WP7.

1.3.6.2 Key Work Undertaken and Results Achieved

Animal studies

All animals were housed in filter top cages with a 12 hrs dark/light cycle, and had unlimited access to food and water throughout the study duration. To assess the effective dose of PA-MSC in the prevention of diabetic

glomerulosclerosis, male C57BLKS Leprdb/db type2 diabetic mice (Taconic, Ry, Denmark) that underwent uninephrectomy (1K) at the age of 6 weeks, were used for this study.

Task 5.1: To evaluate the effective dose of PA-MSc in the prevention of progressive diabetic nephropathy

In order to determine optimal dose of injection, we choose two doses i.e. low dose of 0.25×10^6 cells and high dose of 1×10^6 cells per mouse along with vehicle control. Functional and clinical parameters of diabetic nephropathy analysis clearly demonstrated low dose is sufficient in improving the diabetic phenotype.

Task 5.2 To compare the efficacy of CD362+, CD362- and PA-SSc in the prevention of diabetic nephropathy

After determining 0.25×10^6 cells as the optimal dose from task 5.1 experiments, we further investigated the efficacy of ORBCEL-M with CD362⁻ and PA-MSc cells. GFR, proteinuria and glomerular sclerotic scores were significantly improved in all the groups of mice compared to the vehicle control group, however, CD362⁺ cell treated mice showed more improvement compared with CD362⁻ cell treated mice .

Task 5.3 Biodistribution of ORBCEL-M

Our results based on the detection of human DNA (ALU) sequences in mouse tissues show hardly any cells migrating to the kidney or other organs (other than the lungs). These results lead us to speculate that the effects of ORBCEL-M observed in our earlier experiments might be due to the paracrine nature of these cells. Hence, these results prompt us to further investigate to identify the systemic effector molecules.

Task 5.4 Identifying a biomarker of intrarenal MSC bioactivity

Since our biodistribution studies clearly demonstrated the paracrine nature of ORBCEL-M, we decided to analyse the systemic effector molecules of MSC which could also function as biomarkers. We analyzed spleen, kidney, and blood samples for various immune cells such as T-cells and its sub classes, B-cells and macrophages at the same time points as those of the biodistribution studies and decided to probe the db/db mouse blood samples to identify a potential systemic biomarker of MSC bioactivity.

Results of this task demonstrated increased levels of CD4⁺FoxP3⁺ regulatory T-cells in the spleen after ORBCEL-M injection. In this tissue, we also observed that B-cells were down regulated until 24 hrs after MSC injection and activated macrophages were also maintained at low levels over time except for a significant increase at 6 hrs. In contrast, in blood, T-regulatory cells were reduced over time. For the kidney sample analysis no changes in these immune cells were found. Since upregulation of FoxP3⁺ T-cells in the spleen was clearly demonstrated, we initially hypothesized that following up the levels of FoxP3⁺T-cells in the blood could act as biomarker to track down the activity of injected MSC. However, our results did not show any significant increase in the levels of FoxP3 regulatory T-cells in blood samples over time, thus further studies will be needed to fully elucidate the role of these immune regulatory cells as potential biomarkers of SSC bioactivity.

Task 5.5 Evaluate SSC therapy effects on kidneys of the models used by other REDDSTAR partners

In addition we also received kidney samples from the STZ and Db/Db mouse models from REDDSTAR partner CHT and from the IR and DR mouse models from REDDSTAR partner QUB. Mice which were used in cardiomyopathy studies (both STZ and db/db)(CHT,Berlin) showed higher sclerosis and treatment with CD362⁺ MSC (ORBCEL-M) reduced sclerotic scores significantly. Treatment with CD362⁻ MSC did not improve the scores. Analysis of mice used in retinopathy studies (STZ and Citrate) (QUB, Belfast) revealed no significant damage to kidneys with IR of eyes and no significant improvement with treatment with CD362⁺ MSC.

1.3.6.3 Conclusion

A low dose (0.25 million) of PA-MSc at a 4 month timepoint was more effective in preventing proteinuria and glomerulosclerosis compared to a high dose (1 million) of MSC in 1K db/db mice. 0.25 million PA-MSc significantly reduced the Hb1Ac levels and increased the serum c-peptide levels. Consistently, the animals receiving 0.25 million PA-MSc showed reduced blood glucose levels at the end of the treatment. However, the body weights were identical in all groups. In addition, those mice showed suppressed proteinuria and were associated with the highest levels of GFR.

Single intravenous injection of CD362⁺ MSC (ORBCEL-M) at a 4 month timepoint was more effective in preventing diabetic renal injury as compared to all other treatments (vehicle, CD362⁻ MSC and PA-MSC). Mice injected with CD362⁺ MSC showed higher levels of GFR and showed reduced proteinuria as compared to vehicle treated group. This was associated with less renal inflammation and prevention of glomerulosclerosis. But this effect was very moderate as compared to the mice that injected with PA-SSC or CD362⁻ SSC. Though CD362⁺ and CD362⁻ MSC are equally effective in improving albuminuria, GFR and glomerulosclerosis, there are some trends that suggest better efficacy of CD362⁺ MSC.

Biodistribution studies indicated that the mechanism of action of MSC administration was more likely paracrine in manner as the majority of ORBCEL-M cells were found in lung tissue and the cells did not migrate to the kidney itself. This is a very interesting observation in that we found significant beneficial effects of ORBCEL-M administration on kidney tissue 2 months after a single cell administration, even though the cells were not present in the kidney tissue to exert direct effects and in fact had been cleared from the mouse circulation by this time.

We conclude here that blood sample analysis for FoxP3+T-regulatory cells is not a suitable biomarker for the efficacy of MSC systemic administration and perhaps other immune cells may serve as better biomarkers of MSC bioactivity.

Analysis of the kidneys harvested from other models of diabetes complications by network partners did not reveal a consistent therapeutic effect of ORBCEL-M on the kidney. While some efficacy was seen in the db/db mouse model of diabetic cardiomyopathy this was not seen in the models of STZ-induced diabetes or citrate-induced injury. This may relate to the specific natures of injury in each model.

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1.3.7 WP6 Diabetic Ulcers

1.3.7.1 Introduction

A common complication of diabetes mellitus is prolonged and incomplete wound healing caused by compromised angiogenesis (blood vessel formation), diminished cell recruitment, lack of growth factors, prolonged inflammation and impaired formation of collagen matrix. There is a critical clinical need to develop therapies for non-healing diabetic foot ulcers as 15-25 % of people with diabetes will develop foot ulceration, and diabetic foot ulceration

precedes amputation in 80 % of cases (1). Topically applied mesenchymal stromal cells (MSCs) may provide a novel treatment for diabetic wound healing. We have previously shown that topically applied allogeneic MSCs in a collagen matrix improve wound healing by increasing percentage wound closure and augmenting angiogenesis (2). Increased percentage wound closure is a highly relevant wound healing endpoint as it can be measured in a non-invasive fashion and is the primary endpoint in human clinical trials on diabetic ulceration.

1.3.7.2 Key Work Undertaken and Results Achieved

The preclinical efficacy and subsequent safety of topical and a combination topical/IV application of MSCs to the treatment of dermal wounds was assessed as part of REDDSTAR WP6. The pre-clinical efficacy of a novel MSC cell type (CD362⁺ MSCs, ORBCEL-M) was assessed in head to head studies with CD362⁻ and regular plastic adherent MSCs (PA-MSCs). Within this WP potential mechanisms of MSC mediated action by CD362⁺ MSCs were also investigated.

The alloxan induced diabetic rabbit ear ulcer model was the model used to assess the pre-clinical efficacy of MSC in REDDSTAR WP6. This established model is used in diabetic wound healing research and closely resembles stimulated repair of chronic human diabetic ulcers. Three MSC cell types (CD362⁺, CD362⁻, PA-MSC) were evaluated in a head to head study. The results indicated that wounds treated with CD362⁺ MSC mixed with an Excellagen[®] collagen scaffold demonstrated increased percentage wound closure when compared with untreated wounds or Excellagen[®] treated wounds alone. The CD362⁺ MSCs (ORBCEL-M) significantly augmented the wound healing potential of Excellagen[®] and were also shown to increase blood vessel formation. Both surface density and length density of blood vessels were significantly increased and radial diffusion distance significantly decreased in wounds treated with Excellagen[®] plus CD362⁺ MSC when compared to untreated wounds after one week. These stereological measures provide an indication that there was increased angiogenesis (blood vessel formation) in wounds treated with Excellagen[®] plus CD362⁺ MSCs. Thus, the application of CD362⁺ MSCs in an Excellagen[®] matrix may lead to a new therapeutic product with improved wound healing potential coupled with a reduced healing time.

The results generated in period 1 of REDDSTAR WP6 were compiled into a report, which was sent to the independent REDDSTAR clinical trial selection committee in Copenhagen. This independent committee assessed data from several pre-clinical partners in order to decide which of the REDDSTAR complications would go to clinical trial. Following review of the available data, the REDDSTAR clinical trial selection committee announced that Diabetic Ulcers and CD362⁺ SSC (ORBCEL-M) were the chosen complication and cell type to proceed to the REDDSTAR clinical trial. As part of the clinical trial application to the Danish regulator (the DKMA), it was determined that a more long term topical toxicology study of the administration of CD362⁺ MSC in an Excellagen[®] collagen scaffold to the pre-clinical rabbit wound model would be required. This topical toxicology study was initiated and was the focus of WP6 work in Period 2. The overall objective of this in house topical toxicology study was a safety assessment within NUIG of the topical application of human mesenchymal stem cells (CD362⁺ MSCs) seeded in an Excellagen[®] scaffold to a New Zealand white rabbit ear ulcer model. Two groups of healthy rabbits were assessed per cell treatment and were monitored post treatment for study periods of 1 week and 10 weeks respectively. Haematology, clinical chemistry assessment, and histopathologic analysis of ear ulcers were performed. Selected tissues were analysed for a biodistribution study. The overall aim was to demonstrate that no negative clinical or pathological effects were observed in the CD362⁺ MSC treated animals when compared to control animals over the study periods. Results indicated that there were no treatment related findings on final body weight, body weight gain, food consumption or clinical signs. All haematology parameters fell within normal ranges, all animals were healthy throughout the study and there were no gross or microscopic pathology findings of note. In histopathological analysis by an independent reviewer, no tumour formation or any abnormal wound healing was observed in any animals treated with CD362⁺ SSCs. Biodistribution analysis indicated the presence of human DNA at only the site of administration (ear wound) in the 1 week and 10 week studies. These results indicated that the topical administration of CD362⁺ MSC to rabbit ear ulcers was well tolerated with no treatment related findings of note observed.

This in house NUIG topical toxicology study and full detailed results from the efficacy studies have been incorporated into the REDDSTAR Investigators Brochure (IB), which was submitted to the Danish Medicines agency (DKMA) in June 2016 as part of the REDDSTAR clinical trial authorisation (CTA) application. This clinical trial application is now approved by the DKMA (Aug 2016).

More recent investigations in WP6 in period 3 were focused on elucidating the mechanism of action (MOA) by which MSCs mediate diabetic wound healing. Following very promising pre-clinical results achieved with bone marrow derived CD362⁺ MSCs (also termed Syndecan-2 positive MSCs) detailed above, in period 3 WP6 MOA efforts focussed on investigating Syndecan-2, as there is evidence to suggest potential for this gene having a significant role in the MOA behind MSC mediated diabetic wound healing. Syndecan-2 is a member of the four member family of heparan sulfate proteoglycans, which have been considered to have important roles in cell adhesion, migration, growth factor and cytokine signalling (3). As a result initial MOA work in WP6 was performed to elucidate the mechanism of MSC mediated wound healing in DM through in vitro experimentation to investigate the influence of Syndecan-2 on factors such as and TGF- β , NF-kB, IL-6, IL-8 and endothelial cell activation markers E-selectin, ICAM and VCAM. Subsequently an in vivo study was performed to examine the effects of overexpressing or knockdown of the Syndecan-2 molecule in MSCs. Further in vivo studies are planned to fully elucidate the effect of Syndecan-2 on wound healing in the alloxan induced diabetic rabbit ear ulcer model.

1.3.7.3 Conclusion

REDDSTAR WP6 has assessed the safety and efficacy of novel CD362⁺ and CD362⁻ MSC compared to PA-MSC and have examined their ability to simultaneously control hyperglycaemia and ameliorate diabetic complications, in this case diabetic ulcers. Administration of human derived MSCs to a rabbit model of diabetic ulceration is safe as no adverse effects were found in the animal model. WP6 has determined that all three cell types are efficacious in diabetic wound healing but that that in 'head to head' studies CD362⁺ cell type (ORBCEL-M) is the best cell type to proceed with further studies on wound healing. In the diabetic wound model assessed here there was no effect on hyperglycaemia across both studies. Route of administration was also assessed (topical versus combination topical/IV treatment) and it was determined that while there was a slight increase in percentage wound healing in the combination treatment group this increase was not significant.

The central objective of REDDSTAR has been to assess the effect of MSC therapy in each of six pre-clinical models of diabetic complications, with the ultimate aim of progressing positive pre-clinical results from one complication to a clinical trial at the Steno Diabetes Center in Copenhagen. The REDDSTAR team has successfully evaluated the effects of 3 MSC cell types (PA-MSC, CD362⁺ and CD362⁻) in each model of diabetes and an independent panel at the Steno diabetes centres chose Diabetic Ulcers and CD362⁺ MSC as the chosen complication and cell type to proceed to the REDDSTAR clinical trial. A detailed CTA application was submitted to the DKMA and National Committee on Health Research Ethics (NVK) in June 2016 to perform a phase 1b trial of the topical application of CD362⁺ MSCs in a collagen scaffold (REDDSTAR ORBCEL-M) to non-healing diabetic ulcers in human patients.

The CTA application was approved by DKMA and the NVK on 03rd Aug and 29th Aug 2016 respectively. Securing both the DKMA and NVK approvals was a significant achievement and milestone for the project and ensures the phase 1b clinical trial is in line with Danish medical and ethical standards. Now that approval has been secured from both the DKMA and the NVK, the phase 1b clinical trial of the treatment of diabetic ulcers with CD362⁺ MSC (REDDSTAR ORBCEL-M) can commence, and the REDDSTAR consortium is committed to implementing the trial in early 2017 funded by REDDSTAR partner Orbsen Therapeutics Ltd.

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1.3.8 WP7 Diabetic Bone Fractures

1.3.8.1 Introduction

Patients with T1DM develop early onset osteopenia or osteoporosis. This results in an increased risk of fracture as well as poor bone healing and regeneration after injury. Fractures in diabetic patients take up to 163% longer to heal than non-diabetic fractures.

Analogous bone abnormalities are observed in animal models of T1DM that also exhibit bone loss due to reduced bone formation. Accordingly, the bones of these animals have decreased mechanical integrity, decreased mineral content and inferior fracture healing potential.

The bone marrow of diabetic rodents has increased adiposity (tendency to form more Adipocyte or fat cells), indicating irregular populations of MSCs compared to normal bone marrow. MSCs from diabetic rodents have also reduced colony forming and differentiation potential, resulting in reduced ability to contribute to bone fracture repair. Thus we wished to evaluate if exogenous administration of healthy MSCs would impact on diabetic bone fracture healing.

The overall aims of the studies in WP7 were:

- To evaluate the potential of purified, characterized MSC to improve diabetic fracture repair.
- To clarify the optimal dose, timing and administrative route of MSC to improve diabetic fracture healing in view of clinical translation.
- To investigate underlying mechanisms of inhibited diabetic fracture repair and the contribution of therapeutic MSCs.

1.3.8.2 Key Work Undertaken and Results Achieved

Initial efforts focused on developing a murine model of diabetic fracture healing. This model was then used to assess the efficacy of MSCs to enhance fracture healing, profiling the influence of cells administered at different time points following fracture and optimizing the number of cells administered to have the greatest impact on fracture repair. The methods employed to assess the influence of MSCs on fracture repair include microCT and four point bending analysis. It was found that the administration of MSCs does indeed support diabetic fracture repair.

With an optimized acute model of murine fracture repair, three different sources of MSCs were compared in vivo. Here we used MSCs isolated using traditional methods from the bone marrow, MSC selected based on their expression of proteins on the cell surface and MSCs isolated due to the absence of specific protein expression on the cell surface. It was determined that the addition of cells did result in a trend of increased mechanical integrity in de novo bone formation; however, there was no statistically significant difference between each cell type.

Further, we created, validated and utilized a sensitive, quantitative assay to detect the presence of human DNA in murine tissue samples, thereby indicating the migration and retention of administered MSCs. Unique qPCR primers were developed at NUIG that purposely bound to the human-specific region of the repeating ALU gene. A SOP was developed and distributed to all REDDSTAR partners allowing for comparable characterization of human MSC distribution in each in vivo model. When used to profile cell distribution upon administration to a fracture, it was determined that xenotransplanted cells were retained in low numbers for 48-72 hours, with complete elimination of the human cells by day 7.

Finally, to understand the mechanism by which MSCs were supporting fracture repair, in vitro and in vivo experiments were conducted. To ascertain the role of a BMP-family member in MSC-stimulated fracture repair, the effect of a BMP supplementation of hyperglycaemic MSC chondrogenesis was assessed. MSCs grown in hyperglycaemic conditions were found to have a trend to decreased capacity to differentiate. Supplementation with a BMP restored capacity to differentiate at a comparable level to normoglycaemic MSCs. In vivo, knocking down

MSC expression of that same BMP resulted in an inhibition of the MSCs ability to improve callus remodelling. The exact mechanism by which this BMP influences MSC-mediated fracture repair remains under investigation.

1.3.8.3 Conclusion

Therefore, at the completion of WP7 we have successfully evaluated the potential of purified, characterized MSC to improve diabetic fracture repair and identified the optimal dose and timing of MSCs administration to stimulate diabetic fracture repair. We have created a novel, quantitative assay to identify and quantify human cells upon administration to rodent and lapine models of diabetes. This assay has supported the REDDSTAR clinical trial regulatory submission. Finally, we have examined one underlying mechanism of inhibited diabetic fracture repair which has provided interesting results and targets for further studies.

1.3.9 WP8 Data Management

1.3.9.1 Introduction

WP8 (Data Management) was designed to facilitate the sharing, management and backup of the REDDSTAR pre-clinical research data emanating from WPs 1 to 7.

The key objectives of WP8 were:

1. To gather multi-disciplinary data from each pre-clinical partner in a secure shared data repository.
2. To agree the data to be collected and how it was to be analysed and processed.
3. To configure the data platform to meet the specific needs of this project and this team.
4. To install and validate the software platform.
5. To support the pre-clinical research partners in their use of the data platform throughout the project.

Study Vault-RS (a cloud-based secure data management system designed and developed by PT) has been used to meet REDDSTAR's data management requirements.

1.3.9.2 Key Work Undertaken and Results Achieved

Study Vault is a secure cloud-based data management service that helps researchers, clinicians, and analysts to share research data securely online. Study Vault was designed specifically for high-security, controlled-access, anonymised, and shared research data storage and analyses. Working with the REDDSTAR data partners, a bespoke customisation of Study Vault was developed – Study Vault-RS – to meet the specific requirements of the REDDSTAR project.

Technologists at PT worked intensively with all of the relevant data partners (pre-clinical partners including CHT, LMU-M, NUIG, ORB, QUB, and UPORTO) to modify Study Vault to suit their data management needs. Subsequent to agreement of the data platform configuration, PT technologists configured Study Vault-RS, to meet data requirements, feature requirements, policy/authorisation requirements, and non-functional requirements. On completion of the configuration process, Study Vault-RS was installed and validated (usability assessment and technological validation). Key metrics considered included user acceptance, usability, uptime, apdex, load time, throughput, response time, error rate, CPU usage, memory, database transactions, and browser capabilities. The main benefits identified included: resource sharing, user acceptance, consistency in reporting, auditing and logging, scalability, fault tolerance, ease of use, security, manageability, application monitoring (server and application automatically monitored to ensure system performs as expected), good database performance, and 24-7 availability.

Strong communication and a defined workflow for requirements gathering and feedback elicitation underpinned the design and subsequent ratification of Study Vault-RS. A continuous delivery approach was implemented to ensure that the development of Study Vault-RS was as dynamic as possible. A technological framework was used which enabled the WP8 team to make updates to the live Study Vault-RS system while ensuring only very brief periods of down time. The work delivered enhancements to the Study Vault system and functionality (e.g. inclusion of up-

loaders to handle spread-sheets from 96-well plate outputs), verification of the security and trust model, new data harmonisation workflows, as well as alteration to PT’s technological workflow (via continuous development).

The roll out of Study Vault-RS to pre-clinical data partners provided them with the capability to easily share and compare results. The sharing of data using Study Vault-RS facilitated the analysis and cross correlation of data and helped inform research decisions.

Upload of data, adjustments and customisations of the data platform continued throughout the project, as required. The table below provides a brief overview of the datasets received and uploaded over the course of the entire project from kickoff to completion.

Table 1 Overview of Datasets uploaded to Study Vault

WP	Study	Datasets		
WP1	ORB – Incidence Study	Weight, blood glucose, and HbA _{1c} for: T2 Incidence Study		
WP2	UPORTO - Diabetic Neuropathy PA-SSC efficacy Trial	Weight, glycemia and HbA _{1c} for: <ul style="list-style-type: none"> STZ STZ+PA_105_1x106 STZ+PA_109_1x106 STZ+PA_110_1x106 STZ+S2+_H11_1x106 STZ+S2+_H11_2x106 STZ+S2+_H11_4x106 	Weight, glycemia and HbA _{1c} for: <ul style="list-style-type: none"> STZ+PA_H11_2x10⁶ STZ+S2_H11_2x10⁶ 	
WP4	CHT - Diabetic cardiomyopathy Intravenous application of MSC	Weight, blood glucose and HbA _{1c} for: <i>Dbdb + i.v. application</i> <ul style="list-style-type: none"> Control+PBS db/db Mice + PBS db/db Mice + MSC wt db/db Mice + S2-/MSC orb- db/db Mice + S2+/MSC orb+ 	<i>STC + IV application</i> <ul style="list-style-type: none"> Control+PBS STZ + PBS STZ + MSC wt STZ + MSC wt STZ + S2+/MSC orb+ 	
WP5	LMU-M Diabetic nephropathy	Weight and blood glucose for Vehicle, Low Dose, High Dose groups		
WP6	NUIG wound closing (NUIG Rabbit Study)	Weight, HbA _{1c} and % wound closure for: Topical administration of MSC (task 6.1) <ul style="list-style-type: none"> Includes % closure of No Treatment, Excellagen, Excellagen + S2- cells, Excellagen + S2+ cells, Excellagen + WT MSCs 	Rabbit toxicology study (Haematology measurements)	
WP7	NUIG Fractures (NUIG Fracture Study)	Weight, blood glucose, and HbA _{1c} for: <ul style="list-style-type: none"> Saline 500000 cells 1 million cells 	<i>Blood glucose for PASSC Optimization</i> <ul style="list-style-type: none"> Saline 250,000 cells 500,000 cells 	<i>Weight, blood glucose and HbA_{1c} for:</i> Head to Head Trial <ul style="list-style-type: none"> Saline WT S2+ S2-

The rapid evolution of cloud-based services such as Amazon Web Services and Digital Ocean hosting meant that the Study Vault solution was forced to evolve, itself, in order to remain available and fully supported. An online ‘snaglist’ was maintained across the Study Vault team, which recorded, managed and resolved any issues as they were identified. Technical upgrades over the course of the project included:

- Improved performance: Response time and memory usage were improved by updating the internal mechanisms for dealing with data.
- Updated UI for querying data: Table filter to show all values for any one factor. The data shown was also updated to include useful metadata (e.g. date/timestamp).
- Team design upgrade: Easier setup of team roles i.e. administrator customisation of access for teams outside their organization (e.g. edit, no edit).
- Encryption – Real-world testing successful: Different encryption keys installed for each project on the Study Vault system.

Finally, the Exploitation Workshop (21st October 2014) and subsequent Exploitation Plan (D12.2), explored IP, commercial and research opportunities and issues for project outputs, including the enhanced Study Vault offering. A key success was the roll out of Study Vault in a second FP7 project (MERLIN - Stem cells and therapeutics for tackling liver disease, EU FP7-HEALTH-2013-INNOVATION-1, Grant no. 602363), which commenced in February 2014.

The following screen-shots illustrate Study Vault-RS at the time of drafting of this report.

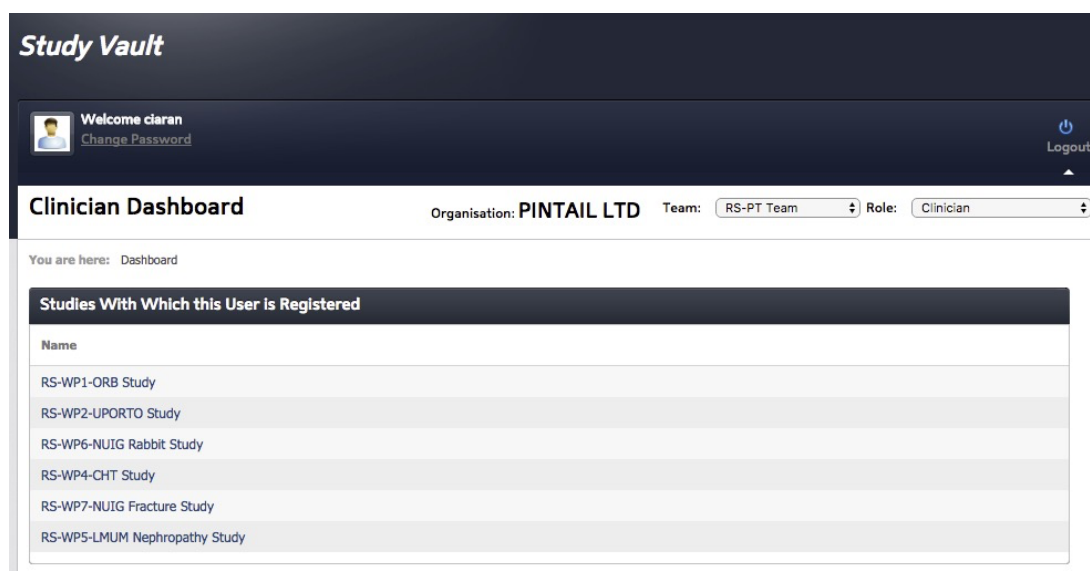


Figure 2 initial screen, with list of studies for which data is held in SV

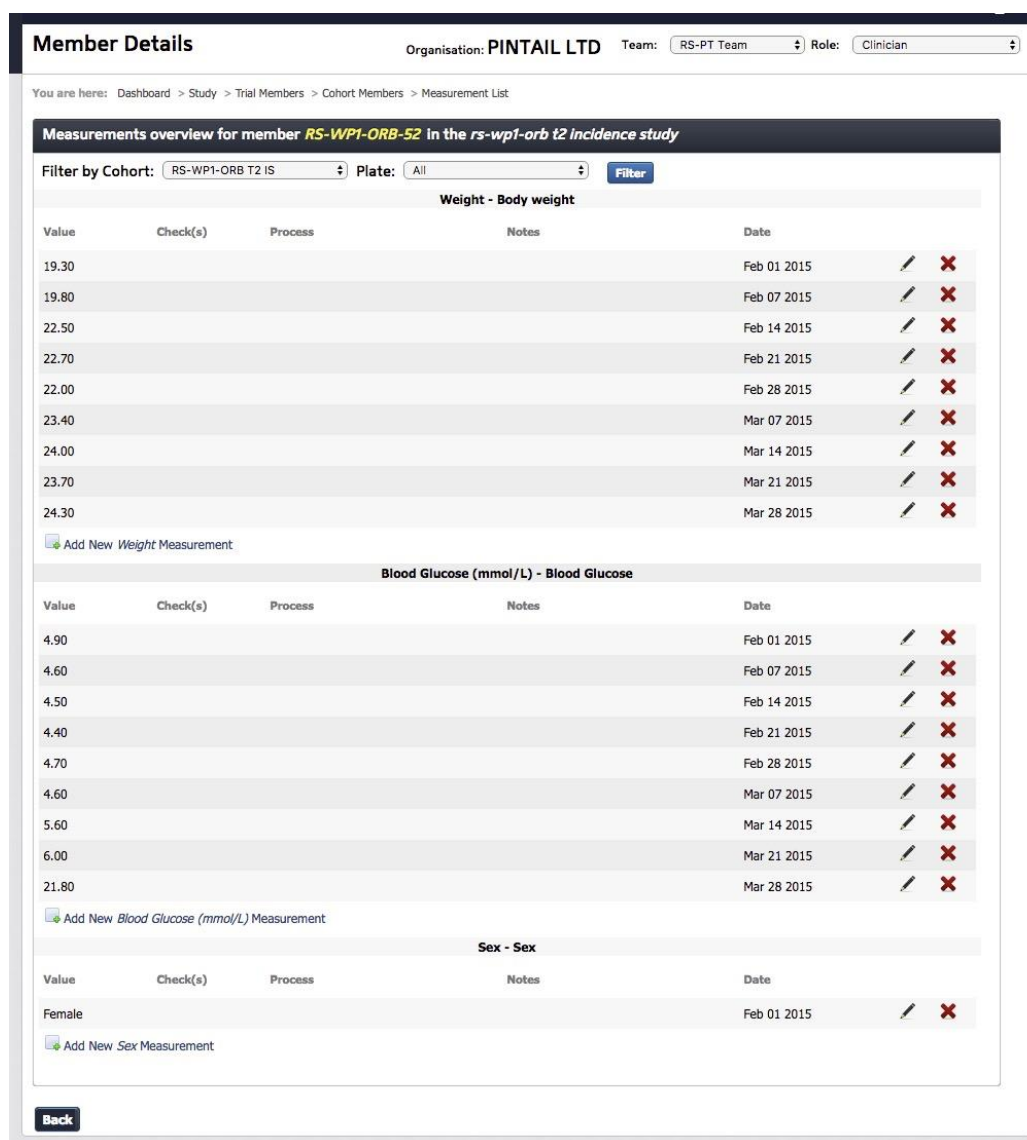


Figure 3 Examples of measurements held for a member of a study

1.3.9.3 Conclusion

WP8 has facilitated the sharing, management and backup of the REDDSTAR pre-clinical research data emanating from WPs1 – 7. In order to analyse and cross correlate the data for several different complications (the markers, measurements, and factors which are recorded by the researchers), the data needed to be accessible and harmonised across the consortium. Study Vault-RS has provided the framework for comparison of stromal cell therapy across diabetic complications (weight, blood glucose, HbA1C, C-peptide, insulin – each represented using agreed metrics, i.e. units, number of decimal places, etc.). The data gathered by the pre-clinical partners also represents an important resource for further secondary analyses, research, and publications.

Study Vault has delivered secure data management, user management and import/export functionality, which is customised and configured to meet the needs of REDDSTAR. It provides web-based access to study information, intervention details, and research data. Data are encrypted both at rest (in the database) and in transit (using an encrypted SSL link between the server and the user’s browser). Partners can access, update, and query information in house while sharing data with the project partners. Study Vault is deployed on the cloud (hosted on Amazon’s Elastic Compute Cloud, EC2) and accessed by partners via a web application (via login at <https://www.studyvault.eu>). As such, resources are provisioned on a dynamic and on-demand basis over the web, meaning that the platform responds dynamically to the demands of the user (e.g. user access and data load).

This approach to data management and sharing improves the utility and credibility of this data within the project (through agreed data metrics) and provides support for future research endeavours. The virtual research community established during REDDSTAR will be enhanced by access to shared data through Study Vault-RS, which will continue to be available to the data partners and will facilitate further exploitation and dissemination after the project ends, for at least three years (i.e. to end 2019).

Through collaboration with REDDSTAR, PT has been able to ratify, customise, expand and improve the Study Vault platform. This has resulted in a web application that is accepted by users, useful in a scientific context, technologically validated, and implemented in such a way that it can be continually updated to meet the needs of different users. The enhanced version of Study Vault has already been rolled out in EU FP7 project MERLIN and will provide a useful tool for other research projects in the future.

1.3.10 WP9 GMP Cell Production

1.3.10.1 Introduction

The primary intent of WP9 was to develop and install a cell sorter based on OWL's microfluidic valve technology for the isolation of MSCs in a process compliant with subsequent therapeutic use. Validation of functionality and implementation of the technology would be demonstrated by installation and GMP manufacturing process development at LUMC, leading to regulatory approval for GMP production of ORBCEL-M to be administered in the REDDSTAR clinical trial on non-healing diabetic ulcers.

1.3.10.2 Key Work Undertaken and Results Achieved

The initial approach taken in WP9 was to develop a new device for cell sorting, integrating the capabilities of OWL's existing Nanosorter with Miltenyi's multilaser analytical platform the MACSQuant. OWL built and delivered a prototype device. However, the new technology needed to be tested and improved, so in order to allow time for this, the REDDSTAR team determined that MSC product development for the proposed REDDSTAR trial should proceed using a more traditional magnetic separation strategy. LUMC, Orbsen and Miltenyi collaborated in several technology evaluation activities, determined the most successful strategy and proceeded with GMP MSC manufacture.

Several cell labelling reagents and labelling protocols were investigated in addition to various combinations of negative and positive selection strategies. Initially the incorporation of CliniMACS separation technology was attempted but since cell yield proved insufficient, a switch was made to LS MACS separation, combining multiple positively selected cell fractions for most favourable yield. The finally developed procedure includes the generation of 3 positively selected cell fractions that are combined, provided that the target cells in each cell fraction are at least 10 times enriched. This selection process was integrated with an existing GMP cell expansion strategy and with a newly developed formulation and packaging process to finally result in an alternative integrated GMP process for the production of REDDSTAR ORBCEL-M, including 3 sequential successful production test runs in the GMP facility. This approach allowed the REDDSTAR Investigational Medicinal Product Dossier (IMPD) to be finalised and submitted with the REDDSTAR clinical trial application. The finalisation of the IMPD required collection of a substantial amount of additional (proprietary) data from several suppliers of raw materials and excipients as well as the production of bespoke antibody batches for the MSC isolation process of the future clinical GMP production of REDDSTAR ORBCEL-M. Additional validation data were also generated for the filling process of the syringes used as primary packaging.

The CTA application for the REDDSTAR study was approved by both the Danish Medicines Agency (DKMA) and the National Committee on Health Research Ethics (NVK) in August 2016. The consortium is committed to delivering the trial after the end of REDDSTAR (in early 2017). The integrated GMP process for the production of REDDSTAR ORBCEL-M developed in WP9 will be used to produce cells for use in the clinical trial.

Notwithstanding that Tyto will not be used for the REDDSTAR trial itself, OWL and Orbsen continued to evaluate and develop the Tyto instrument for the isolation of MSCs from human bone marrow, as delivering this new technology constituted a significant project objective. We were able to improve instrument reliability issues dramatically and quickly moved on to improve fundamental instrument performance. We installed an early version of Tyto at Orbsen, transferred our feasibility protocol successfully and the team in Orbsen has since been using the system for research work related to the MSC product.

The final Tyto REDDSTAR process is designed for isolation of very rare cells, using two stages in the sorting process, one in which the instrument is configured to maximize the yield of the desired cells starting with a concentrated cell suspension, followed by a second sort which strives to purify output of the first sort.

Between June of 2015 and May of 2016, 21 bone marrow sorts were performed on Tyto, the first 6 at OWL in Santa Barbara CA and the rest at Orbsen in Galway. As is commonly understood, human bone marrow from healthy donors varies considerably in many respects, including with regard to the total cellularity of the harvest and frequency of the MSC population of interest. The details of Tyto's efficiency isolating these cells likewise varies, but in all cases the cells of interest were harvested with purities meeting REDDSTAR's specifications. Most importantly, in several cases, the isolated cells were apparently 100% capable of forming colonies in culture, our most significant in vitro assay for the health and potential of the cells. This is a novel finding.

Our most important limitation remains the yield of the process, which we are continuing to work on, both with regard to mechanical constraints of the fluid path, and the details of the sort valve operation.

1.3.10.3 Conclusion

Within REDDSTAR we have developed and validated a novel benchtop cell sorter, the MACSQuant Tyto. The MACSQuant® Tyto™ is a high-speed, 10-parameter microchip-based cell sorter in a fully closed cartridge system, which offers rapid, multi-colour flow sorting with high purity and ease of use. This was the first real world implementation of a novel technology that has far reaching value for cell purification in clinical and research use generally and cell therapy specifically. Cell purification is an important capability in therapeutic cell characterization, enhanced potency, minimization of confounding effects and reliable clinical performance. Alternative purification technologies have limitations with regard to sample/environment isolation, process control and utility in expertise-limited manufacturing environments. The MACSQuant Tyto mitigates these limitations, thus broadly enabling progress in cell therapy development, and is now commercially available with early units being installed globally.

REDDSTAR has resulted in an investigational Advanced Medicinal Product (REDDSTAR ORBCEL-M) and an approved clinical protocol for the treatment of Diabetic ulcers. Work in WP9 has played a significant role in delivering those achievements.

1.3.11 WP10 Clinical Trial

1.3.11.1 Introduction

The overall objectives of WP 10 (Clinical Trial) were:

1. To provide scientific and clinical recommendations related to the animal models effect on glycaemia and how the cell treatments may affect glucose metabolism as well as the development and progression of complications.
2. To provide consultancy to the REDDSTAR advisory board related to selection, design, initiation, and implementation of Phase 1b trials.
3. To design and implement the clinical trial protocols for REDDSTAR Phase 1b Clinical Trial.
4. To oversee and manage the regulatory compliance considerations for initiating the Phase 1b REDDSTAR Cell Therapy trial.

The central objective of REDDSTAR has been to assess the effect of mesenchymal stromal cell (MSC) therapy in each of six pre-clinical models of diabetic complications, with the ultimate aim of progressing positive pre-clinical results from one complication to a clinical trial at the Steno Diabetes Center in Copenhagen.

The REDDSTAR team successfully evaluated the effects of 3 MSC cell types (PA-MSC, CD362⁺ and CD362⁻) in each model of diabetes (WPs 2 to 7 above) and generated exciting pre-clinical results showing the positive effects of CD362⁺ MSC (now called ORBCEL-M) on blood glucose, kidney disease, neuropathy and wound healing, in addition to immunomodulatory effects in a pre-clinical model of diabetic cardiomyopathy, and beneficial effects in ischaemic and diabetic retinopathy.

1.3.11.2 Key work undertaken and results achieved

Based on these positive pre-clinical results an independent panel at Steno Diabetes Center selected 'Treatment of Diabetic Ulcers with ORBCEL-M' for progression to a Phase 1b clinical trial in REDDSTAR. Subsequent clinical efforts in WP10 were focussed on the generation, review and management of the regulatory compliance documents necessary for the REDDSTAR clinical trial application to the Danish Medicines Agency (DKMA) and the Danish National Committee on Health Research Ethics (NVK) for a clinical phase 1b study on the treatment of diabetic ulcers with CD362⁺ MSC (ORBCEL-M). Regulatory and ethical approval of these documents was a pre-requisite prior to the initiation of the REDDSTAR 01 clinical study on non-healing diabetic foot ulcers. Consultation was undertaken with the REDDSTAR advisory board related to selection, design, initiation and implementation of the clinical trial and recommendations for moving forward were developed.

REDDSTAR partners NUIG, ORB, LUMC and Steno actively collaborated on the REDDSTAR clinical trial submission to the Danish regulator the DKMA, and to the NVK.

The CTA application was approved by both DKMA and the NVK on 03rd Aug and 29th Aug 2016 respectively to conduct the REDDSTAR study, so the next steps are the performance of the phase 1b clinical study in non-healing diabetic ulcers, and plans for same are being finalised between the REDDSTAR partners at present. Securing both the DKMA and NVK approvals was a significant achievement and milestone for the project and ensures the phase 1b clinical trial is in line with Danish medical and ethical standards. Securing the approval took just two months - an exceptionally short time for any regulatory approval - reflecting the completeness of our application documents and the regulator's perception of the safety and value of the trial. In addition, the speed and completeness of this regulatory review is a testament to the remarkable work ethic and team spirit that the REDDSTAR consortium has forged during our program. This speed of this approval is the more remarkable when consideration is given to the complexities of the REDDSTAR trial & therapy which is -

1. a "First in Man" clinical trial,
2. an allogeneic therapy,
3. combining both cell therapy and a biomaterial,
4. for the treatment of non-healing diabetic foot ulcers.

The work in WP10 directly addresses REDDSTAR specific aim #4: To carry out a Phase 1b Clinical Trial of CD362 therapy of a chosen complication. Now that approval has been secured from both the DKMA and the NVK, the phase 1b clinical trial of treatment of diabetic ulcers with CD362⁺ MSC (ORBCEL-M) can commence, and the REDDSTAR consortium plans are being finalised for this currently. Plans are also in hand for future studies. In terms of future studies, based on the success of the CTA application, the consortium is already exploring the possibility of executing a randomized, double-blind, placebo controlled Phase 2 trial of ORBCEL-M in patients with non-healing DFUs. This trial would require screening about 400 patients to meet a goal of enrolling and treating between 100 and 150 patients. As a result of the REDDSTAR project, the consortium's knowledge of GMP manufacturing has significantly increased and REDDSTAR has provided the foundation and roadmap needed for further implementation of novel ORBCEL-M MSC based therapies into clinical trials.

1.3.11.3 Conclusion

In conclusion, WP10 has focused on overseeing and managing the necessary regulatory compliance documents in preparation for the Phase 1b clinical trial and full regulatory and ethical approval has been achieved for the REDDSTAR 01 clinical study. Consultation undertaken with the REDDSTAR advisory board related to selection, design, initiation and implementation of the clinical trial and plans for moving forward with future Phase 1 to Phase 2 trials have been developed.

1.3.12 Main S&T Results: Conclusion

The REDDSTAR project began on 1 November 2012 and finishes on 31 October 2016. Within REDDSTAR we have successfully evaluated the effects of 3 MSC cell types (CD362⁺ (ORBCEL-M), CD362⁻ and PA MSC) in different models of diabetes. The team has generated exciting pre-clinical results, demonstrating the positive effects of our novel MSC cell type (ORBCEL-MTM) on blood glucose, kidney disease, neuropathy and wound healing. In addition, our work has demonstrated the immunomodulatory effects of ORBCEL-MTM in a pre-clinical model of diabetic cardiomyopathy and beneficial effects in ischaemic and diabetic retinopathy. We have also investigated potential mechanisms of action of these MSC in relation to specific diabetic complications.

Based on our positive pre-clinical results, an independent panel at the Steno Diabetes Centre in Copenhagen selected treatment of diabetic ulcers with ORBCEL-MTM for further exploration in a Phase 1b clinical trial. The Danish Medicines Agency (DKMA) and Danish National Committee on Health Research Ethics (NVK) recently approved the REDDSTAR Clinical trial authorisation application (CTA) to conduct this study, and the trial is planned to initiate early in 2017.

In addition within REDDSTAR a new cell sorter, The MACSQuant[®] Tyto[™] (OWL Biomedical/Miltenyi Biotec) has been developed and ratified with the aim of mitigating all of the sample integrity risks associated with conventional droplet sorters. The MACSQuant[®] Tyto[™] is a closed, single-use consumable, environmentally controlled, multi-laser cell sorter that aims to meet future therapeutic regulatory requirements.

The next step for the consortium will be the conduct of the phase 1b clinical study into non-healing diabetic ulcers. Plans for the study are being finalised and it is anticipated that the trial will commence in early 2017. Securing both the DKMA and NVK approvals was a significant achievement and milestone for the project and ensures the phase 1b clinical trial is in line with Danish medical and ethical standards. Successful completion of the Phase 1b trial (demonstrating safety and indicating efficacy), would provide the evidence needed to progress to a more advanced trial, with a larger patient population, which would focus on the efficacy of the treatment.

As indicated above we have found that administration of MSC (and in particular ORBCEL-MTM) has had beneficial effects on more than one diabetic complication. In addition to positive effects on diabetic ulcers, there was a strong indication at the 18 month review meeting that this cell type had significant beneficial effects in a mouse model of diabetic kidney disease (DKD). Preclinical data developed by REDDSTAR partner LMU in collaboration with partner ORB confirms that intravenous single dose administration of human ORBCEL-MTM can restore kidney function, reduce proteinuria, limit lymphocyte activation, reduce glomerular sclerosis and attenuate the expression of inflammatory cytokines IL6 and TNF in a db/db uni-nephrectomised model of DKD. In this model we also found positive effects of the cell therapy on blood glucose levels. Our REDDSTAR data is consistent with a variety of independent preclinical studies on the effects of stromal cells in pre-clinical models of DKD.

Based on these positive pre-clinical results for DKD, REDDSTAR partners together with new collaborators successfully obtained EC H2020 funding to follow on and investigate the clinical effects of ORBCEL-MTM in a 4 site clinical trial in 48 patients with DKD. The ambitious new research project called NEPHSTROM (Novel Stromal Cell Therapy for Diabetic Kidney Disease) began in May 2015 and is a collaboration of 11 European partners (www.nephstrom.eu) and builds on the successful pre-clinical research carried out in REDDSTAR.

We believe that REDDSTAR results have the potential to significantly impact the management and treatment of diabetes, with relevance for clinicians, researchers in diabetes and regenerative medicine, drug/pharmaceutical companies, patients and for the general public. Our aim has been to improve the treatments available for diabetic patients, and to enhance their health and quality of life. Ultimately it is hoped that such improvements can lead to a reduction in the public health costs associated with diabetes and related complications.

1.4 Impact, dissemination and exploitation.

1.4.1 Impact and exploitation

1.4.1.1 Background

Diabetes Mellitus (DM) is considered a major global health problem at present. Both type 1 (T1DM) and type 2 (T2DM) cause blood glucose levels to increase above normal and current treatment strategies (e.g. insulin) frequently result in suboptimal glycaemic control, and may cause life-threatening hypoglycaemic (low blood glucose) reactions. Over time, poorly controlled diabetes can lead to glucose toxicity, inflammation and a variety of serious secondary tissue complications, including heart disease, ulcerating wounds, blindness, amputations, kidney disease, and nerve damage. In fact, DM is the leading cause of blindness, end-stage renal disease, and a variety of debilitating neuropathies in the western world. T2DM is a public health concern and projections of its future effects are alarming. According to the International Diabetes Federation, the worldwide prevalence of diabetes in 2015 is 415 million and is set to raise to 642 million by 2040 (International Diabetes Atlas 7th edition 2015; www.diabetesatlas.org/). The emerging pandemic is driven by the combined effects of aging population, increased urbanisation, changes in lifestyle with an increased caloric and dietary fat intake, rising levels of obesity and inactivity.

REDDSTAR has sought to address the challenges presented by DM in a novel way, through the exploration of stromal cell therapies to control glycaemia and treat diabetic complications. The multi-disciplinary “whole patient” approach taken for the treatment of DM in REDDSTAR is unique.

1.4.1.2 What REDDSTAR has achieved?

Within REDDSTAR we have successfully evaluated the effects of 3 MSC cell types (CD362⁺ (ORBCEL-M), CD362⁻ and PA-MSC) in different models of diabetes. The team has generated exciting pre-clinical results, demonstrating the positive effects of our novel MSC cell type (ORBCEL-MTM) on blood glucose, kidney disease, neuropathy and wound healing. In addition, our work has demonstrated the immunomodulatory effects of ORBCEL-MTM in a pre-clinical model of diabetic cardiomyopathy and beneficial effects in ischaemic and diabetic retinopathy. We have also investigated potential mechanisms of action of these MSC in relation to specific diabetic complications.

Based on our positive pre-clinical results, an independent panel at the Steno Diabetes Centre in Copenhagen selected treatment of diabetic ulcers with ORBCEL-MTM for further exploration in a Phase 1b clinical trial. The Danish Medicines Agency (DKMA) and Danish National Committee on Health Research Ethics (NVK) recently approved the REDDSTAR clinical trial authorisation application (CTA) to conduct this study, and the trial is planned to initiate early in 2017. Securing both the DKMA and NVK approvals was a significant achievement and milestone for the project and ensures the phase 1b clinical trial is in line with Danish medical and ethical standards. A successful trial for the treatment of diabetic ulcers, demonstrating safety and indicating efficacy, would provide the evidence needed to progress to a more advanced trial, with a larger patient population, which would focus on the efficacy of the treatment.

As indicated above, we have found that administration of MSC (and in particular ORBCEL-MTM) has had beneficial effects on more than one diabetic complication. For example, preclinical data developed by REDDSTAR partner LMU in collaboration with partner ORB confirms that intravenous single dose administration of human ORBCEL-MTM can restore kidney function, reduce proteinuria, limit lymphocyte activation, reduce glomerular sclerosis and attenuate the expression of inflammatory cytokines IL6 and TNF in a db/db uni-nephrectomised model of diabetic kidney disease (DKD). In this model we also found positive effects of the cell therapy on blood glucose levels. Based on these positive pre-clinical results for DKD, REDDSTAR partners together with new collaborators successfully obtained EC H2020 funding to follow on and investigate the clinical effects of ORBCEL-MTM in a 4 site clinical trial in 48 patients with DKD. The ambitious new research project called NEPHSTROM (Novel Stromal Cell Therapy for Diabetic Kidney

Disease) began in May 2015 and is a collaboration of 11 European partners (www.nephstrom.eu) and builds on the successful pre-clinical research carried out in REDDSTAR.

We have already demonstrated the potential benefits of ORBCEL-M™ for the treatment of diabetes and diabetic complications and further research opportunities are being pursued to develop this potential. However, there is also evidence that suggests that stromal cells can be beneficial in the treatment of other chronic non-communicable diseases, e.g. where there is inflammation, auto-immunity, oxidative stress, and/or high blood pressure. A positive clinical validation of ORBCEL-M™ in the REDDSTAR trial (and in our follow on NEPHSTROM project) would provide an excellent precedent that will facilitate clinical trials in other non-communicable diseases such as lupus, coronary artery disease, heart failure, asthma and other conditions.

Within REDDSTAR we have also developed and validated a novel benchtop cell sorter, the MACSQuant Tyto. The MACSQuant® Tyto™ is a high-speed, 10-parameter microchip-based cell sorter in a fully closed cartridge system, which offers rapid, multi-colour flow sorting with high purity and ease of use. This was the first real world implementation of a novel technology that has far reaching value for cell purification in clinical and research use generally and cell therapy specifically. Cell purification is an important capability in therapeutic cell characterization, enhanced potency, minimization of confounding effects and reliable clinical performance. Alternative purification technologies have limitations with regard to sample/environment isolation, process control and utility in expertise-limited manufacturing environments. The MACSQuant Tyto mitigates these limitations, thus broadly enabling progress in cell therapy development, and is now commercially available with early units being installed globally.

Finally, as part of our work in REDDSTAR we also developed Study Vault-RS, a bespoke data management platform which enabled multi-national researchers, clinicians, and analysts to share research data securely online. The platform provides an easy-to-use, 24-7 available environment with which to manage and share pre-clinical research data. The enhanced version of Study Vault developed in REDDSTAR has already been rolled out in another EU FP7 project (MERLIN grant agreement no 602363) and will provide a useful tool for other research projects into the future.

1.4.1.3 Future Exploitation

REDDSTAR's Exploitation Plan maximises the impact and research value of the project by mapping out the commercial and academic research development of the project results (outlined above) into the future.

Plans and possibilities for future research exploitation by the partners to build on their results in REDDSTAR include:

- As indicated previously, the results of pre-clinical experiments in NUIG on the treatment of diabetic ulcers with CD362⁺ MSC's are being progressed to a phase 1b clinical trial on diabetic patients. There are plans to commence the trial in early 2017. Partners are committed to taking this next essential step for the development of possible new therapies for non-healing, neuro-ischaemic diabetic foot ulcers (with potential adaptation for other conditions). It is hoped that following the phase 1b trial, phase 2/3 clinical trials can be initiated.
- Pursuing H2020 funding opportunities for further research – note the diabetic nephropathy data from REDDSTAR has already led to further research in H2020 project NEPHSTROM.
- Assessing potential for a new COST action focused on research into diabetes complications in tandem and related therapeutic innovations.
- Continuing with REDDSTAR's focused publications strategy (which helps advance the partners' research agendas and raise profile in industry, which may also further commercial objectives).
- PT will continue to develop Study Vault as a cloud-based data management platform that enables multi-national researchers, clinicians, and analysts to share research data securely online. Study Vault is already in use in other EU research projects, building on the experience gleaned from REDDSTAR (e.g. MERLIN).
- During the project, NUIG developed a novel, sensitive, quantitative assay to accurately quantify the number of human cells in a mouse, rat or rabbit organ. This assay enables the detection of migration and retention of administered human MSC. The methodology has already been reviewed and approved by the Irish regulatory

authority in support of a separate Phase I clinical trial application in NUIG for critical limb ischaemia. There is a publication in press to describe the assay. NUIG will continue to make use of and to disseminate this innovation.

- REDDSTAR is closely aligned with UPORTO's strategic research agenda and UPORTO will continue to build on the work undertaken in the project e.g. the collaboration has already resulted in a spin-off project "*Stromal stem cells therapy to prevent diabetes-induced changes in the central nervous system*" funded by FCT, the Portuguese national funding agency for science, research and technology.
- REDDSTAR is well aligned with the research agenda of partner CHT. In addition to publications already achieved, further publications are planned by CHT e.g. a cardiac-specific manuscript demonstrating the efficacy of CD362⁺ cells to reduce diabetic cardiomyopathy after intravenous application, a paper on the db/db mouse as model of diabetic cardiomyopathy, including a timeframe study.
- QUB's participation in REDDSTAR has already allowed it to further its research agenda through significant dissemination opportunities, conference presentations and public events. Future plans include a retina-specific manuscript which will demonstrate the efficacy of the CD362 cells for direct ocular delivery. In the light of very positive data from the REDDSTAR research in relation to diabetic retinopathy, QUB has also been successful in a joint funding application with NUIG under the SFI-DEL Ireland/ N. Ireland partnership programme 2015.
- LUM's future research plans include leading a work package in the Horizon 2020 project NEPHSTROM to further investigate the mechanism of action and immune response of ORBCEL-M™ in diabetic kidney disease (DKD) in conjunction with NUIG, ORB, LUMC and PT. This work has directly resulted from exciting results generated in REDDSTAR. REDDSTAR LMU trained endocrinologist Dr Junhui Xie, also hopes to secure Chinese funding to work on MSC upon her return to China and LMU plans a long-standing academic collaboration with their Chinese colleague in the future on diabetic nephropathy. REDDSTAR has shaped LMU's interest in other diabetes complications too; LMU are now expanding their interest beyond the kidney and focussing on diabetes as a systemic disease, which is a significant development of their research agenda.
- NUIG's future research plans include the further exploration of progenitor cell-based fracture repair in a diabetic model. A number of publications are planned based on REDDSTAR preclinical results and a number of additional funding applications are envisaged. In addition, NUIG will coordinate and manage the Horizon 2020 project, NEPHSTROM.
- REDDSTAR has led to collaborations by Steno with Bispebjerg Hospital (Copenhagen). It is anticipated that this new collaboration will continue for future ventures. REDDSTAR has also increased focus on the treatment of diabetic foot ulcers at Steno and on the potential for the development of novel therapies.
- Participation in REDDSTAR has enabled LUMC to further develop their leading role in Advanced-Therapy Medicinal Product (ATMP) development, production and clinical application. Through REDDSTAR LUMC has introduced new technology in their translational work flow. The MACS Quant Tyto technology advanced in REDDSTAR will not only support the generation of improved, more homogeneous, MSC products but is also instrumental for further development of multiple other ATMPs in the LUMC hospital.
- The REDDSTAR Virtual Research Community (VRC) was established during the project to ensure that the collaborative relationships established in REDDSTAR endure and provide a foundation for further research and development. A Memorandum of Understanding (MoU) was agreed by the partners to establish the terms of interaction, use and responsibility of the Virtual Research Community, and to cement partners' desire to work together as possibilities arise in the future. The core vision of the legacy is a new perspective – a multidisciplinary, broadly-focussed view of diabetes/complications research which provides a set of supports and resources for teams to identify and pursue new challenges which build on the existing data, clinical samples, success and results of earlier work.

Future plans and possibilities for commercial exploitation by the partners to build on their results in REDDSTAR include:

- Further developing the unique multi-disciplinary offering developed in REDDSTAR, so industry can simultaneously and efficiently test/analyse/interpret the efficacy and safety of 'products' across 6 complications of diabetes in well-validated disease models. The group will also investigate potential for additional studies e.g. neurodegenerative aspects of type 2 diabetes, Alzheimer's, Parkinson's disease.
- Exploring options to collaborate with industry.
- OWL will continue to develop the ground-breaking MACSQuant® Tyto cell sorter. REDDSTAR resulted in redevelopment of OWL's testing, quality metrics and some design constraints, which has resulted in a product much better suited to a broad cell therapy and basic research global market.
- ORB will continue to develop its ORBCEL cell therapy product for market, building on the platform provided by REDDSTAR. As a result of ORB's participation in REDDSTAR, the company is now partnered with 25 independent and global leaders in MSC therapy development, across the EU and North America, for the validation, development and commercialization of ORBCEL products in a number of fields, including diabetic, auto-immune and inflammatory conditions.

1.4.1.4 Who will benefit?

It will be clear from the above that REDDSTAR has delivered some exciting new knowledge with potential significance for the treatment of diabetic ulcers and other diabetic complications (we have also demonstrated positive effects of ORBCEL-M™ on kidney disease and neuropathy, as well as the immunomodulatory effects of ORBCEL-M™ in diabetic cardiomyopathy and beneficial effects in ischaemic and diabetic retinopathy). In addition, there are strong indications that stromal cells can also be beneficial in the treatment of other chronic non-communicable diseases, with impact on (among others) inflammation, auto-immunity, oxidative stress, and high blood pressure. Possible conditions which may be of interest include lupus, coronary artery disease, heart failure and asthma.

REDDSTAR has advanced the state of the art and knowledge and has opened up a number of avenues for further research. REDDSTAR has already benefited the partners in the consortium in the pursuit of their own research and commercial agendas. However the advances made will also benefit other researchers in diabetes and regenerative medicine and the research community as a whole. The clinical trial which we will deliver post REDDSTAR will provide a reference and roadmap for other clinical translations, facilitated by the GMP-compliant, well-characterised cells which the project has delivered.

The delivery of advanced cell therapies will inevitably effect industry, in particular drug/pharmaceutical companies, as industry will ultimately play a role in the further development, scaling and delivery of these treatments. Quality, consistency and regulation have been significant constraints on the growth of the sector to date. It is precisely these areas where REDDSTAR can have its greatest commercial impact, as the project brings exciting new sorter technology and a powerful new tool for cell isolation and characterisation – together, these have the potential to provide major impetus to the industry.

Our belief that the project has the potential to significantly impact the management and treatment of diabetes, clearly points to a tangible clinical benefit and a direct impact on clinicians and their patients. In particular we believe that REDDSTAR will result in enhanced health and quality of life for diabetic patients, with knock-on benefits for their families and networks. These clear societal benefits may be matched by economic ones, as better health among patients with T2DM may result in increased economic productivity and reduced public health costs.

Finally, the project is aligned with key public health and research policy objectives and has the potential to inform future policy by demonstrating improvements in patient health through clinical trials that can be translated into healthcare savings.

1.4.2 Dissemination

The REDDSTAR team undertook a comprehensive dissemination programme over the course of the project. Our target audience included life science researchers, academics, clinicians, drug development companies, other projects and initiatives, patient representative groups, the media and the general public. We also engaged directly with students and young people, in an educational role. Our aim has been to raise awareness about the project, our research and results. Below we describe the dissemination activities undertaken including online, peer reviewed publications, conference presentations, organised events and project materials.

1.4.2.1 Website and online

The REDDSTAR website (<http://www.reddstar.eu/>) was launched on 3rd October 2012 and has played a central role as a public dissemination tool and means of communication throughout the project. Different sections of the website are aimed at different audiences, including scientists, industry, researchers, media and the general public. The website includes the following pages:

- Home page <http://www.reddstar.eu/> - a general introduction to the project.
- Project Page <http://www.reddstar.eu/project/> - a more detailed look at REDDSTAR, with subpages focused on Innovation and Societal Value; Patients; Industry; Steno Diabetes Centre Videos and Useful Links.
- Scientific Page <http://www.reddstar.eu/scientific/> - dedicated scientific area including subpages regarding Phases of the Project, Project Publications, Related Projects and Research; Related Policy and Legislation, REDDSTAR Mid-Project Report and the REDDSTAR Exploitation Workshop.
- News Page <http://www.reddstar.eu/news/> – regular updates on the project and related developments, together with subpages dedicated to the Stem Cells Revolutions Event, the Diabetes It’s Complicated Event and Press Releases and Media.
- Partners Page <http://www.reddstar.eu/partners/#all/1/list> - details of each partner in the consortium with links to partner websites.
- Contributors Page <http://www.reddstar.eu/contributors/> - information about people from outside of REDDSTAR who are making contributions to the project.
- Contact Page <http://www.reddstar.eu/contact/> – contact details for the Coordinator and online form which can be used to engage with the project team.

The main website is in English, but in the first 6 months the website content was translated from English to Dutch (<http://www.reddstar.eu/nl/>), German (<http://www.reddstar.eu/de/>) and Portuguese (<http://www.reddstar.eu/pt-pt/>). Some pages also appear in Danish.

Visitors to the website have grown over the course of the project. Relevant details are set out in Table 2.

Table 2 Basic Website Statistics Taken From <http://reddstar.eu/awstats/> .

Period	Unique Visitors	No of Visits
1 Nov 2012 – 31 Oct 2013	17,090	26,346
1 Nov 2013 – 31 Oct 2014	13,571	26,484
1 Nov 2014 – 31 Oct 2015	18,785	50,891
1 Nov 2015 – 31 Oct 2016	20,170	39,729

REDDSTAR

Home Project Scientific News Partners Contributors Contact

Welcome to REDDSTAR
 The REDDSTAR consortium is a network of diabetes specialists, regenerative medicine researchers, biotech industrialists and clinicians, supported by an experienced project management team.

Partners

REDDSTAR – Repair of Diabetic Damage by Stromal Cell Administration [More Information](#)

Project Area

Visit this section to find out more about our Project and the innovations and benefits we aim to achieve. We have also included some links to other websites relating to diabetes that may be useful. This section of our website includes information that may be of interest to the general public (our public area).

[Click Here](#)

Scientific Area

Visit this section for technical information relating to our Project including a summary of the phases of the Project, details of Project publications, information about related research and links to relevant policy and legislation.

[Click Here](#)

News

REDDSTAR Final Project Leaflet
October 28, 2016

New REDDSTAR Publication in Neurobiology of Disease
October 20, 2016

Reddstar partners published in Neuropharmacology
July 7, 2016

REDDSTAR Plenary in Galway
April 27, 2016

REDDSTAR reaches over 200 dissemination activities!
March 29, 2016

Twitter

The REDDSTAR team are pleased to issue the final project flyer summarising key results. Access the flyer here: t.co/ADDmatCA25
Time ago 14 Days via Twitter Web Client

New REDDSTAR paper on pain modulation from the brain during diabetic neuropathy: Neurobiology of Disease
t.co/N7aWqQeELU
Time ago 22 Days via Twitter Web Client

Follow [@reddstaru](#) on twitter.

Partners

Pages

- Home
- Project
 - Innovation and Societal Value
 - Patients
 - Industry
 - Steno Diabetes Center Videos
 - Useful Links
- Scientific
 - Phases of the Project

Project Information

Copyright – FP7 REDDSTAR 2012

Figure 4 Screenshot from REDDSTAR homepage <http://www.reddstar.eu/>

In addition to the website, REDDSTAR has established and maintained a lively social media presence. By the end of the project REDDSTAR had 324 likes on Facebook and 261 followers (racking up a total of 952 tweets).



Figure 5 Screenshot from REDDSTAR Facebook Page

1.4.2.2 Media

The partners have engaged with the media over the course of the project, as a conduit to reach their target audience. Press releases have been issued by all partners (e.g. to announce their involvement in REDDSTAR) and the website includes a special media subpage. Coverage in the media has included articles in the mainstream print media (e.g. Irish Times 19/11/2012 and 2/8/2014, Belfast Telegraph 14/2/2013; Irish Daily Mail 14/2/2013; Connacht Tribune 22/08/2014, Irish Independent 01/08/2014); radio and t.v. (e.g. Portuguese TV, Ulster TV, Radio EU, Galway Bay FM Radio,) and numerous news updates online (e.g. on <http://www.medicalnewstoday.com>; <http://www.medpagetoday.com>; <http://www.news-medical.net/> and <http://www.sciencedaily.com>).

1.4.2.3 Project Materials

We developed different project materials over the course of REDDSTAR to raise awareness about our work. We produced a 'REDDSTAR Industry brochure', which was designed and distributed to a wide cohort of academic, industry and patient organisations (more than 200 brochures were posted with letters of introduction and invitations to collaborate). Patient education factsheets were also created for the six diabetes complications addressed in REDDSTAR. These were co-branded with Diabetes Ireland, enabling wide circulation e.g. on the Diabetes Ireland website. REDDSTAR promotional materials were distributed at a number of conferences and events (e.g. 1,200 delegates received REDDSTAR promotional postcards in their delegate packs at the ISCT Conference in Paris April 2014, materials were also distributed at the 30th Congress of the International Society for Advancement of Cytometry CYTO conference in Glasgow on 26th – 30th June 2015).

Home Project Scientific News Partners Contributors Contact

Patients

REDDSTAR is not recruiting patients for a diabetes complication clinical trial.

Please view our informational factsheets for patients.

Bone and the four types of cells

Diabetes mellitus (DM) is a metabolic disorder associated with several complications, including impaired healing. Bone, an important structural tissue, is affected in the disease condition. Identifying during fracture healing processes. Fracture healing is a complex, multi-phased process that requires bone structure and biological properties of its repair cells. It has been well documented that DM, a systemic disease causes impaired healing time by 2-3 times the normal rate.

Bone and the four types of cells

Bone consists of a hard, mineral matrix, which is a porous structure, where an open space for red blood cells and blood vessels is found. Osteocytes, osteoblasts, osteoclasts, and stem cells are the four types of cells that make up the bone. Osteocytes are the most numerous cells in bone, and they are responsible for maintaining the bone's structure. Osteoblasts are responsible for forming new bone, and osteoclasts are responsible for breaking down old bone. Stem cells are responsible for producing new bone cells.

Diabetes mellitus affects the bone by affecting the function of these four types of cells. Osteocytes are affected by hyperglycemia, which leads to a decrease in their ability to maintain the bone's structure. Osteoblasts are affected by hyperglycemia, which leads to a decrease in their ability to form new bone. Osteoclasts are affected by hyperglycemia, which leads to an increase in their ability to break down old bone. Stem cells are affected by hyperglycemia, which leads to a decrease in their ability to produce new bone cells.

Diabetic retinopathy

The retina is the light-sensitive layer of tissue at the back of the inner eye. It converts light that enters the eye into nerve impulses that are sent to the brain. During diabetes, the retina and the blood supply can be damaged. Over time, poorly controlled blood sugar can cause damage to the small blood vessels in the retina. This damage is called retinopathy. Retinopathy is a complication of diabetes that usually develops in people with type 1 diabetes who have had the disease for at least 10 years. It usually develops in people with type 2 diabetes who have had the disease for at least 20 years.

Other people with early diabetic retinopathy

- People who have had diabetes for a long time
- People who have had diabetes for a long time and who are also obese
- People who have had diabetes for a long time and who are also smokers
- People who have had diabetes for a long time and who are also pregnant

Why you have?

- A long time has passed since you were first diagnosed with diabetes
- You have had diabetes for a long time and you are also obese
- You have had diabetes for a long time and you are also smokers
- You have had diabetes for a long time and you are also pregnant

Diabetic neuropathy

Approximately half of all people with diabetes mellitus develop some degree of neuropathy, a condition that can affect all parts of the body. It is most commonly diagnosed in the feet and hands. Diabetes neuropathy most commonly occurs when a person with diabetes has prolonged levels of high blood sugar levels. Prolonged blood glucose and acute fluctuations of glucose levels can cause damage to the nerves. Nerve damage is most common in the feet and hands, but it can also affect the eyes, stomach, and bladder. Nerve damage can also affect the heart, kidneys, and bladder. Nerve damage can also affect the brain, leading to memory loss, depression, and anxiety. Nerve damage can also affect the skin, leading to dry skin, itching, and blisters.

Other people with early diabetic neuropathy

- People who have had diabetes for a long time
- People who have had diabetes for a long time and who are also obese
- People who have had diabetes for a long time and who are also smokers
- People who have had diabetes for a long time and who are also pregnant

Why you have?

- A long time has passed since you were first diagnosed with diabetes
- You have had diabetes for a long time and you are also obese
- You have had diabetes for a long time and you are also smokers
- You have had diabetes for a long time and you are also pregnant

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March 29, 2016

Bone Repair Factsheet

Retinopathy Factsheet

Neuropathy Factsheet

Search...

Flow cytometry and cell sorting

One technique available to both the clinician and the researcher, flow cytometry is a widely employed in flow cytometry. Cell sorting, also known as fluorescence-activated cell sorting (FACS), is a technique used to separate and analyze cells based on their physical and chemical characteristics. It is a key tool in many areas of biology, including immunology, cell biology, and developmental biology. Flow cytometry is used to identify and analyze individual cells in a population of cells. It is used to study the function of cells and to identify cells that are abnormal. Flow cytometry is also used to sort cells into different populations based on their characteristics. This is done by using a laser to excite fluorescent dyes that are attached to specific markers on the cells. The light emitted by the dyes is then detected and analyzed to identify the cells. Flow cytometry is a powerful tool for studying cells and for identifying cells that are abnormal.

Flow cytometry is performed on a variety of tissues, including:

- Bone marrow
- Blood
- Spleen
- Kidney
- Liver
- Lung
- Pancreas
- Skin
- Tumor

Did you know?

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Diabetic wound healing and foot ulcers

One prevalent complication associated with both type 1 and type 2 diabetes is impaired wound healing. If left untreated, foot ulcers can lead to amputation. Foot ulcers are a major cause of hospitalization and amputation in people with diabetes. They are most commonly found on the bottom of the foot, but they can also occur on the top of the foot, the sides of the foot, and the heels of the foot. Foot ulcers are caused by a combination of factors, including poor blood flow, nerve damage, and foot deformities. Foot ulcers can be prevented by good foot care and regular checkups with a healthcare provider. If a foot ulcer does develop, it should be treated as soon as possible to prevent complications. Treatment may include debridement, antibiotics, and wound care. In some cases, surgery may be needed to remove the ulcer and to improve blood flow to the foot.

Why are diabetic patients more likely to get foot ulcers?

- Poor blood flow to the feet
- Nerve damage in the feet
- Foot deformities
- Poor foot care
- Regular checkups with a healthcare provider

Did you know?

Foot ulcers are a major cause of hospitalization and amputation in people with diabetes. They are most commonly found on the bottom of the foot, but they can also occur on the top of the foot, the sides of the foot, and the heels of the foot. Foot ulcers are caused by a combination of factors, including poor blood flow, nerve damage, and foot deformities. Foot ulcers can be prevented by good foot care and regular checkups with a healthcare provider. If a foot ulcer does develop, it should be treated as soon as possible to prevent complications. Treatment may include debridement, antibiotics, and wound care. In some cases, surgery may be needed to remove the ulcer and to improve blood flow to the foot.

Diabetic neuropathy

Diabetic neuropathy affects the nerves of the body. As the disease progresses, it can lead to a variety of complications, including nerve damage, pain, and loss of sensation. Diabetic neuropathy is most commonly diagnosed in the feet and hands. It is caused by high blood sugar levels, which damage the nerves. Diabetic neuropathy can also affect the eyes, stomach, and bladder. Nerve damage can also affect the heart, kidneys, and bladder. Nerve damage can also affect the brain, leading to memory loss, depression, and anxiety. Nerve damage can also affect the skin, leading to dry skin, itching, and blisters.

Other people with early diabetic neuropathy

- People who have had diabetes for a long time
- People who have had diabetes for a long time and who are also obese
- People who have had diabetes for a long time and who are also smokers
- People who have had diabetes for a long time and who are also pregnant

Why you have?

- A long time has passed since you were first diagnosed with diabetes
- You have had diabetes for a long time and you are also obese
- You have had diabetes for a long time and you are also smokers
- You have had diabetes for a long time and you are also pregnant

Partners

Twitter

The REDDSTAR team are pleased to issue the final project flyer summarising key results. Access the flyer here:...

<https://t.co/ADDmatCA25>

12:15 PM 28, October 2016

New REDDSTAR paper on pain modulation from the brain during diabetic neuropathy: Neurobiology of Disease

<https://t.co/N7aWqQEULU>

12:46 PM 20, October 2016

Prof Isaura Travers (UPORTO) presented a poster at FENS Forum of Neuroscience Copenhagen, 2 July '16. For more see:

<https://t.co/scPG12COMD>

02:37 PM 07, July 2016

Follow @REDDSTAREU 262 followers

Cytometry Factsheet

Diabetic Healing & Foot Ulcer Factsheet

Nephropathy Factsheet

GMP and cell manufacturing

Good manufacturing practice (GMP) is the production and testing process that helps to ensure a quality product. Several countries have regulated that pharmaceutical and medical device companies must follow GMP procedures, and all pharmaceutical and medical device companies must follow GMP procedures. GMP is a set of standards that companies must follow to ensure that their products are of high quality and are safe for use. GMP is a key part of many industries, including pharmaceuticals, food, and medical devices. GMP is used to ensure that products are of high quality and are safe for use. GMP is a key part of many industries, including pharmaceuticals, food, and medical devices. GMP is used to ensure that products are of high quality and are safe for use.

Why are diabetic patients more likely to get foot ulcers?

- Poor blood flow to the feet
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Did you know?

Foot ulcers are a major cause of hospitalization and amputation in people with diabetes. They are most commonly found on the bottom of the foot, but they can also occur on the top of the foot, the sides of the foot, and the heels of the foot. Foot ulcers are caused by a combination of factors, including poor blood flow, nerve damage, and foot deformities. Foot ulcers can be prevented by good foot care and regular checkups with a healthcare provider. If a foot ulcer does develop, it should be treated as soon as possible to prevent complications. Treatment may include debridement, antibiotics, and wound care. In some cases, surgery may be needed to remove the ulcer and to improve blood flow to the foot.

Diabetic cardiomyopathy

Diabetic cardiomyopathy (DCM) is a condition that affects the heart. It is caused by high blood sugar levels, which damage the heart muscle. DCM is most commonly diagnosed in people with type 2 diabetes. It is characterized by a thickening of the heart muscle, which leads to a decrease in the heart's ability to pump blood. DCM can lead to heart failure, which is a condition in which the heart is unable to pump enough blood to the rest of the body. DCM can also lead to arrhythmias, which are irregular heartbeats. DCM can be prevented by good blood sugar control and regular checkups with a healthcare provider. If DCM does develop, it should be treated as soon as possible to prevent complications. Treatment may include medications to improve heart function and to control blood sugar levels. In some cases, surgery may be needed to improve heart function.

Why are diabetic patients more likely to get foot ulcers?

- Poor blood flow to the feet
- Nerve damage in the feet
- Foot deformities
- Poor foot care
- Regular checkups with a healthcare provider

Did you know?

Foot ulcers are a major cause of hospitalization and amputation in people with diabetes. They are most commonly found on the bottom of the foot, but they can also occur on the top of the foot, the sides of the foot, and the heels of the foot. Foot ulcers are caused by a combination of factors, including poor blood flow, nerve damage, and foot deformities. Foot ulcers can be prevented by good foot care and regular checkups with a healthcare provider. If a foot ulcer does develop, it should be treated as soon as possible to prevent complications. Treatment may include debridement, antibiotics, and wound care. In some cases, surgery may be needed to remove the ulcer and to improve blood flow to the foot.

Stromal stem cells

Stromal stem cells (SSCs) can be isolated from bone marrow, the site of their origin, and from other tissues. They are multipotent, meaning they can differentiate into a variety of cell types. SSCs are used to study the development of various tissues and to identify potential therapeutic targets. SSCs are also used to generate cell-based therapies for a variety of diseases, including diabetes. SSCs are a key part of many industries, including pharmaceuticals, food, and medical devices. SSCs are used to ensure that products are of high quality and are safe for use.

Why are diabetic patients more likely to get foot ulcers?

- Poor blood flow to the feet
- Nerve damage in the feet
- Foot deformities
- Poor foot care
- Regular checkups with a healthcare provider

Did you know?

Foot ulcers are a major cause of hospitalization and amputation in people with diabetes. They are most commonly found on the bottom of the foot, but they can also occur on the top of the foot, the sides of the foot, and the heels of the foot. Foot ulcers are caused by a combination of factors, including poor blood flow, nerve damage, and foot deformities. Foot ulcers can be prevented by good foot care and regular checkups with a healthcare provider. If a foot ulcer does develop, it should be treated as soon as possible to prevent complications. Treatment may include debridement, antibiotics, and wound care. In some cases, surgery may be needed to remove the ulcer and to improve blood flow to the foot.

GMP Factsheet

Cardiomyopathy Factsheet


Stromal stem Cells Factsheet

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Figure 6 Screenshot from project patients subpage showing patient materials <http://www.reddstar.eu/project/patients/>

An interview with Dr Stephen Elliman (ORBSEN) was recorded for REDDSTAR promotional purposes and was released on 7th May 2014. The video was added to the EuroStemCell website (www.eurostemcell.org) and Orbsen Therapeutics website (www.orbsentherapeutics.com), and was shared via Facebook. An interview with Dr Martin Ridderstråle, Vice President and Head of Clinic at Steno Diabetes Center was also conducted regarding standard diabetes foot care at the Steno Diabetes Centre (<http://www.reddstar.eu/interview-with-martin-ridderstrale-steno-diabetes-center/>).

In the final weeks of the project the team also compiled the final project flyer, which summarises the aims of the project, the key results achieved and how REDDSTAR has the potential to help patients, clinicians, researchers, pharmaceutical companies, health systems and the general public. The final flyer is available for download from the project website here <http://www.reddstar.eu/reddstar-final-project-leaflet/> and here <http://www.reddstar.eu/news/press-releases/>.



Repair of Diabetic Damage by Stromal Cell Administration

What is REDDSTAR?

Millions of patients with diabetes mellitus in the EU use prescription drugs to control their blood glucose levels. Poor control of blood glucose levels can lead to a number of complications, including: nephropathy (kidney disease), retinopathy (retinal disease), cardiomyopathy (heart disease), neuropathy (peripheral nerve dysfunction), impaired bone repair and wound ulceration. At present, there are few therapeutic options available to control diabetic complications.

REDDSTAR is an EU FP7 project focused on examining whether Mesenchymal Stromal Cells (MSC, from adult bone marrow) can safely control glycaemia and alleviate damage caused by six different diabetic complications.

What has REDDSTAR achieved?

The REDDSTAR project began on 1 November 2012 and finishes on 31 October 2016. As part of our work we have successfully evaluated the effects of 3 MSC cell types in different models of diabetes. The team has generated exciting pre-clinical results, demonstrating the positive effects of a novel MSC cell type (ORBCEL-M) on blood glucose, kidney disease, neuropathy and wound healing. The diabetic nephropathy data from REDDSTAR has already led to further research in H2020 project Nephstrom. In addition, our work has demonstrated the immunomodulatory effects of ORBCEL-M, in a pre-clinical model of diabetic cardiomyopathy. We have also seen its beneficial effect in ischaemic and diabetic retinopathy.

Based on our positive pre-clinical results, an independent panel at the Steno Diabetes Centre

selected treatment of diabetic ulcers with ORBCEL-M for further exploration in a Phase 1b clinical trial. The Danish Medicines Agency (DKMA) and Danish National Committee on Health Research Ethics (NVK) recently approved the REDDSTAR Clinical trial authorisation application (CTA) to conduct this study.

Within REDDSTAR we have also developed and validated a novel benchtop cell sorter, the MACSQuant Tyto. The MACSQuant® Tyto™ is a high-speed, 10-parameter microchip-based cell sorter in a fully closed cartridge system, which offers rapid, multi-colour flow sorting with high purity and ease of use.

How will REDDSTAR help?

Non-healing, neuro-ischaemic diabetic foot ulcers (DFU) are very common and present a significant burden on an individual's health and on healthcare systems. Despite current treatment strategies, there is a high prevalence of non-healing ulcers and amputation. Foot ulcers can affect 12% to 25% of people with diabetes mellitus throughout their lives. Right now there are more than 5 million patients in the US and EU suffering with non-healing DFU. REDDSTAR pre-clinical studies have highlighted the potential for a novel therapeutic product for people suffering with DFU, delivering improved wound healing and reduced healing time. The team is currently exploring options for the conduct of a Phase 1b trial arising from REDDSTAR, to investigate the treatment of diabetic ulcers with ORBCEL-M. The partners are committed to taking this next essential step towards novel clinical treatments for diabetic complications, with potential benefits for millions of patients worldwide.

REDDSTAR has generated exciting and novel results, with prospects far beyond the life of the project. Our findings have the potential to significantly impact the management and treatment of diabetes in the future, with benefits for patients, clinicians, researchers, pharmaceutical companies, health systems and for the general public.



This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 305736. The material presented and views expressed here are the responsibility of the author(s) only. The EU Commission takes no responsibility for any use made of the information set out.

Figure 7 REDDSTAR Final Project Flyer (front page view)

1.4.2.4 Publications

Publications in peer-reviewed journals have been an important part of the REDDSTAR dissemination strategy. There are a total of 20 REDDSTAR publications noted on the EU portal. 8 of these were papers in the proceedings of a conference or workshop, the other 12 publications were in peer reviewed journals:

- *“Mesenchymal stem cell based treatment for microvascular and secondary complications of Diabetes Mellitus”*. Grace C. Davey, Swapnil B. Patil, Aonghus O’Loughlin and Timothy O’Brien. *Frontiers in Endocrinol.* 2014; Jun 6;5:86.
- *“From isolation to implantation: a concise review of mesenchymal stem cell therapy in bone fracture repair”*. Luke Watson, Stephen J Elliman, Cynthia M Coleman. *Stem Cell Research & Therapy* 2014, 5:51 doi:10.1186/scrt439.
- *“Diabetic Bone Fracture Repair: A Progenitor Cell-Based Paradigm. Jessica Hayes, Cynthia Coleman”*. *Current Stem Cell Research & Therapy*, Volume 10, issue 6: 1-11.
- *“Changes in immunological profile of allogeneic mesenchymal stem cells after differentiation: should we be concerned?”* Paul Lohan, Cynthia M Coleman, J Murphy, Matthew D Griffin, Thomas Ritter, Aideen E Ryan. *Stem Cell Research & Therapy* 2014 5:99.
- *“Animal models of kidney inflammation in translational medicine”*. Alexander Holderied, Hans-Joachim Anders. *Drug Discovery Today: Disease Models* Volume 10, Issue 4, Pages e159-e236 (Winter 2013).
- *“Crosstalk between fibroblasts and inflammatory cells”*. S. Van Linthout, K. Miteva, C. Tschöpe. *Cardiovascular Research* Vol. 102/Issue 2 258-269.
- *“Pullulan: a new cytoadhesive for cell-mediated cartilage repair”*. Sarah E Bulman, Cynthia M Coleman, J Mary Murphy, Nicholas Medcalf, Aideen E Ryan, Frank Barry. *Stem Cell Research & Therapy* Vol. 6/Issue 1 pg 34.
- *“Evaluation of Cartilage Repair by Mesenchymal Stem Cells Seeded on a PEOT/PBT Scaffold in an Osteochondral Defect.”* V. Barron, K. Merghani, G. Shaw, C. M. Coleman, J. S. Hayes, S. Ansboro, A. Manian, G. O’Malley, E. Connolly, A. Nandakumar, C. A. van Blitterswijk, P. Habibovic, L. Moroni, F. Shannon, J. M. Murphy, F. Barry. *Annals of Biomedical Engineering* Vol. 43/Issue 9 pgs 2069-2082.
- *“Endovanilloid control of pain modulation by the rostroventromedial medulla in an animal model of diabetic neuropathy”*. M. Silva, D. Martins, A. Charrua, F. Piscitelli, I. Tavares, C. Morgado, V. Di Marzo. *Neuropharmacology*, Volume 107, August 2016, Pages 49–57.
- *“New insights in (inter)cellular mechanisms by heart failure with preserved ejection fraction”*. Sophie Van Linthout and Carsten Tschöpe. *Current Heart Failure Reports* December 2014, Volume 11, Issue 4, pp 436–444.
- *“Pain modulation from the brain during diabetic neuropathy: Uncovering the role of the rostroventromedial medulla”*. Marta Silva, José Tiago Costa-Pereira, Daniel Martins, Isaura Tavares. *Neurobiol Dis.* 2016 Oct 4;96:346-356.
- *“Biodistribution of Locally Administered Human Stromal Cells: Quantitative PCR-Based Detection of Human DNA in Murine Organs”* Creane L, Howard L, O’ Brien T, Coleman C.M.. *Cytotherapy*. In press.

The team intend to publish numerous other papers in the weeks and months ahead based on our REDDSTAR results. Many of these proposed publications are already well underway.

1.4.2.5 Conferences

The REDDSTAR team has also reached its core scientific audience by presenting at numerous key conferences and events. There are a total of 243 dissemination activities entered on the EU portal for the project, many of which relate to conference presentations. A small selection of our key presentations are set out below:

- *“REDDSTAR: Repair of Diabetic Damage by Stromal Cell Administration” and “CD362: A Novel Functional Marker for MSC Isolation and Therapeutic Use”* at REDDSTAR-sponsored session of the International Society for Cellular Therapy 2014 Conference, Paris, 25 April 2014.
- *“Topical Application of ORB1+ Human Mesenchymal Stem Cells Seeded in Excellagen™ Scaffold Augments Wound Healing in a Diabetic Wound Model”* at the 74th Scientific Sessions of the American Diabetes Association (ADA), San Francisco, California, 13 – 17 June 2014.
- *“Translating Mesenchymal Stromal Cell Therapy to the Clinic for Ischaemic Disease and Diabetic Complications: Challenges and Opportunities”* at the 8th UK Mesenchymal Stem Cell Meeting, Galway, Ireland, 29-30 October 2014.
- *“Topical Stem and Progenitor Cell Therapy in a Model of Diabetic Foot Ulceration”* at the Irish Endocrine Society 39th Annual Meeting, Galway, Ireland, 14th November 2014.
- *“Efficacy of Novel Mesenchymal Stem Cell Populations in a Rat Model of Type 1 Diabetes-Induced Neuropathic Pain”* at the Congresso Português de Endocrinologia, 66ª Reunião Anual da SPEDM, Funchal, Portugal, 22nd-25th January 2015.
- *“Local Administration of Non-Diabetic MSCs to Diabetic Femoral Fractures Enhances Callus Remodelling and Deposition of Reparative Bone”* at the Orthopaedic Research Society 2015 meeting, Las Vegas, USA, 28 March 2015.
- *“C362+ stromal stem cell (SSCs) promote revascularisation of the retina in a paracrine manner”* at The Association For Research In Vision And Ophthalmology Annual Congress, Colorado, USA, 3-7 May 2015.
- *“Local administration of non-diabetic MSCs to diabetic femoral fractures enhances callus remodelling and deposition of reparative bone”* at The European Congress of Endocrinology 2015, Dublin, Ireland, (16 –20 May 2015).
- *“Vasoreparative potential of a unique population of stromal stem cells (SSCs) in ischaemic retina”* at The European Association For The Study Of Diabetes, Turin, Italy, 26-28 June/2015.
- *“TA Regulatory Body-Approved Methodology for Localizing and Quantifying Human Mesenchymal Stem Cells Administered to Murine Pre-Clinical Models”* at the TERMIS meeting, Boston, 07 September 2015.
- *“Immune mechanisms of diabetic nephropathy”* at the Munich Nephrology Forum, Munich, Germany, 8 December 2015.
- *“CD362+ mesenchymal stem cells as therapeutic option for treatment of kidney disease in type 2 diabetic LepRdb/db mice”* at ISN-Nexus 2016, Berlin, Germany, 14 April 2016.
- *“CD362+ stromal stem cells (SSCs) stabilize vascular networks through differentiation into pericyte-like cells”* and *“A unique population of stromal stem cells (SSCs) protect against neurovascular dysfunction during diabetic retinopathy”* at The Association For Research In Vision And Ophthalmology Annual Congress 1-5 May 2016, Seattle Washington (abstracts published in Invest Ophthalmol Vis Sci. September 2016, Volume 57, Issue 12.)

1.4.2.6 Other Events

In addition to conference presentations aimed at researchers, academics and industry, the REDDSTAR team has organised and participated in other events, attracting a wider audience from the general public and engaging with schools. Some examples include:

- In conjunction with our plenary meeting in Berlin (5th – 6th November 2013), a public meeting was co-organised with the FP-7 funded project CommHERE. Diabetes DE Board Member Michaela Berger served as a diabetes patient advocate at this REDDSTAR public meeting *Diabetes- Can stem cells help?*
- A series of workshops and debates aimed at secondary school students were organised by REDDSTAR in collaboration with Debating Science Issues (DSI - a cross-border project with 8 biomedical research and science

discovery centre partners). The programme involves 36 schools across the island of Ireland and invites young people to engage in debate on the cultural, societal and ethical implications of advances in biomedical science. The series ran in 2015 and 2016 and each year culminated in the selection of a winning school at a debating final held in Dublin.

- More than 100 people attended the “Diabetes- It’s Complicated” public meeting in Galway, Ireland on 9th September 2014. This public forum was designed to explain what research is doing to help advance treatments for complications of Type 2 diabetes. Speakers included NUI Galway Professor of Medicine, Consultant Endocrinologist and REDDSTAR Coordinator Tim O’Brien; Prof Noemi Lois MD of Queen’s University Belfast; Danielle Nicholson REDDSTAR Dissemination Officer and Dr Cynthia Coleman PhD NUI Galway. The event finished with an interactive question and answer session.
- During Science Week in Ireland (9-16/11 2014), a screening of the 90 minute documentary “*Stem Cell Revolutions- Vision Of The Future*” was held at NUI Galway, followed by a Q&A session involving REDDSTAR partners.
- As part of UNISTEM 2015, (public stem cell event), REDDSTAR partner QUB arranged for an interactive day of talks and hands-on activities for 80 upper secondary students at W5 Science Discovery Centre in Northern Ireland (13 March 2015).

1.4.2.7 Other Projects

We engaged with numerous other projects and initiatives during the course of REDDSTAR through informal contacts, virtual links and structured meetings. The REDDSTAR website includes a special subpage with links to 11 other research projects related to the work of REDDSTAR <http://www.reddstar.eu/scientific/related-projects-and-research/>.

Importantly, the REDDSTAR team played a central role in the EU-MSC2 meeting held in Leiden on 7th and 8th September 2015. REDDSTAR researchers from NUI Galway, Orbsen Therapeutics Ltd., Ludwig-Maximilian’s-Universität München, University of Porto, Queen’s University Belfast, Miltenyi Biotec, Pintail Ltd., Steno Diabetes Center and Leiden University Medical Center attended the meeting. The event brought together researchers from nine EU-funded projects pursuing MSC research. In addition to REDDSTAR, other projects represented at the meeting included Stellar, RETHRIM, REACH, VISICORT, ADIPOA-2, MERLIN, SCIENCE and NEPHSTROM. The event included presentations and interactive panel discussions about key challenges faced when developing MSC therapies (including scientific obstacles, regulatory and ethical issues, technological hurdles and commercialisation barriers). Opportunities for future collaborations and the harmonisation of MSC research in Europe were also discussed. A comprehensive report on the EU-MSC2 meeting in Leiden was released in January 2016. A link to the report is available on the REDDSTAR website <http://www.reddstar.eu/report-on-eu-msc2-2015-meeting-shaping-the-future-of-msc-therapy/>.



Figure 8 REDDSTAR partner Prof. Willem Fibbe of the LUMC opens the EU-MS2 meeting

1.4.3 Conclusion

In REDDSTAR we have achieved some exciting scientific results regarding the positive effects of our novel MSC cell type (ORBCEL-M™) on blood glucose and on different diabetic complications. We have also developed the MACSQuant® Tyto™, a novel technology with far reaching value for cell purification in clinical and research use. We have plans in place for the exploitation of our results through (i) further research and (ii) commercialisation. Most notably, our Phase 1b clinical trial for treatment of diabetic ulcers with ORBCEL-M™ is due to commence in early 2017. We have already achieved success with the kick-off of H2020 project NEPHSTROM – a project based on promising REDDSTAR preclinical results, which will investigate the clinical effects of ORBCEL-M™ in a 4 site clinical trial in 48 patients with DKD.

Throughout the project the REDDSTAR team has followed a targeted dissemination strategy. We have grown the project's presence online through the project website and social media accounts. We have issued project materials (available for download from <http://www.reddstar.eu/>) and have achieved several significant publications (with many more in preparation). Members of the team have presented on our research (and related topics) at numerous key conferences. Finally, we have built a multi-disciplinary network of contacts working in related disciplines, which has added further breadth and depth to our reach.

We believe that our work on dissemination has delivered a tangible awareness of the project's research and results within our target audience, which will yield future opportunities and collaborations. We have also reached out to the public (e.g. through the accessible information made available on our website and through public facing events).

We believe that the work undertaken in REDDSTAR will continue to bear fruit beyond the life of the project and that our results and findings have real potential to impact on the treatment of diabetes and diabetic complications into the future.

1.5 Website and contact details

The REDDSTAR website is at <http://www.reddstar.eu/>

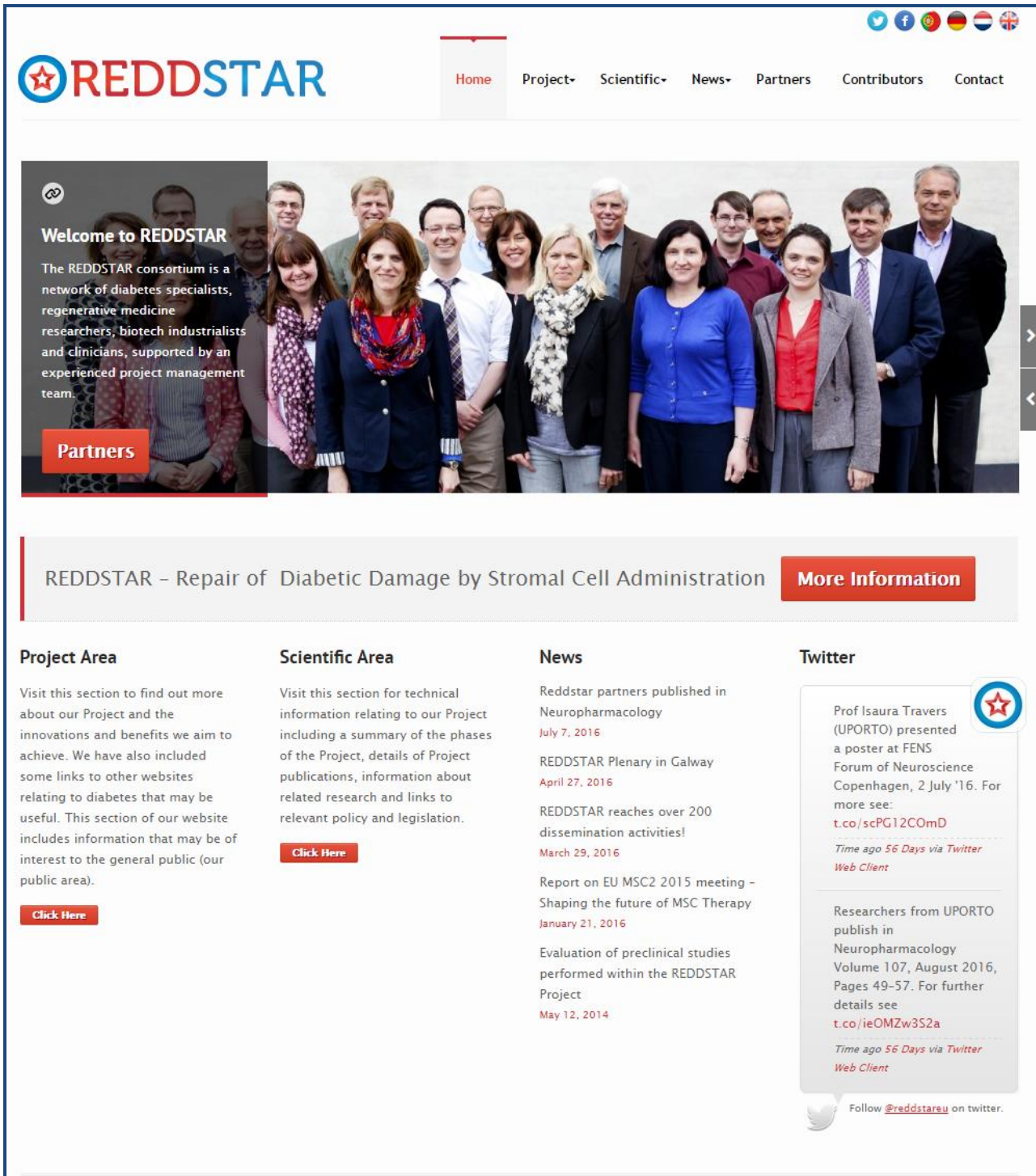


Figure 9 Screenshot from REDDSTAR homepage

REDDSTAR can be contacted via the Project Coordinator:
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The REDDSTAR consortium is a network of diabetes specialists, regenerative medicine researchers, biotech industrialists and clinicians, supported by an experienced project management team.

	<p>NUI Galway</p>
	<p>The Queen's University of Belfast</p>
	<p>Universidade do Porto</p>
	<p>Charité – Universitätsmedizin Berlin</p>
	<p>Ludwig-Maximilians-Universität München</p>



Orbsen Therapeutics Limited



Owl Biomedical Inc



Pintail Ltd



Steno Diabetes Center A/S



LEIDEN UNIVERSITY MEDICAL CENTER

Academisch Ziekenhuis Leiden – Leids Universitair Medisch Centrum (LUMC)

