REDDSTAR

REDDSTAR| Repair of Diabetic Damage by Stromal Cell Administration

Description of work performed to date

The central objective during the **initial 18 months** of REDDSTAR has been to assess the effect of MSC (mesenchymal stem cell) therapy in each of six preclinical models of diabetic complications. The team successfully laid the building blocks for the evaluation of the efficacy of MSC and have progressed towards clinical trial. This included the collection and delivery of samples and analyses of three types of MSC: PA-SSC (plastic adherent, stromal stem cells), $CD362^+$ or $CD362^-$ SSC. CD362 is a cell surface marker which Orbsen Therapeutics' use to select a specific population of cells from the mixture of cells and substances present in the bone marrow. In this first 18 month project phase, procedures for isolation and production of $CD362^+$ and $CD362^-$ SSC from human bone marrow using the MACSQuant Tyto were developed. Positive effects on blood glucose, kidney disease, neuropathy and wound healing have been demonstrated and studies in other diabetic complications are ongoing. An independent panel at Steno selected **Treatment of Diabetic Ulcers with $CD362^+$ SSC for progression to a Phase 1b clinical study**.

Main results achieved in each REDDSTAR Work Package (WP):

SSC Platform- Orbsen Therapeutics Ltd (WP1) provided MSC to all pre-clinical studies and is assessing if human $CD362^+$, $CD362^-$ and PA-SSC can safely reverse streptozotocin (STZ)-induced hyperglycaemia in STZ-treated NOD/SCID mice.

In **Diabetic Neuropathy- University of Porto** (WP2) effects of MSC (PA-SSC and $CD362^+$) were tested in an animal model of T1DM. Metabolic parameters typical of the disease were not affected by intravenous injection of MSC but behavioural signs of diabetic neuropathy were improved after injection of $CD362^+$SSC. Results indicate that

effects of the CD362$^+$ SSC are probably derived from a peripheral effect and not from central actions.

In **Diabetic Retinopathy- Queen's University Belfast** (WP3) efficacy of MSC were assessed in animal models of ischaemic and diabetic retinopathies. Local administration of human CD362$^+$ cells into the eyes of mouse pups under hypoxic conditions significantly reduced avascular area of the retina. However, intravitreal delivery of human MSC into adult mice eyes produced an acute immune response.

In **Diabetic Cardiomyopathy- Charite- Universitatsmedizin Berlin** (WP4), MSC and particularly CD362$^+$ SSC administration resulted in a systemic immunomodulation, leading to less circulating cells expressing pro-inflammatory TNF-α, pro-fibrotic TGF-ß, and less apoptotic T regulatory cells. Also, immunomodulatory effects of CD362$^+$ may contribute in reducing cardiac fibrosis.

In **Diabetic Nephropathy- Ludwig-Maximilians-Universitat, Munich** (WP5) CD362$^+$, CD362$^-$ SSC and PA-SSC are being assessed in long-term db/db models of T2DM diabetic nephropathy. A single dose of MSC was sufficient to reduce blood glucose, increase serum C-peptide, reduce proteinuria and increase glomerular filtration rate (GFR) in this mouse model.

Diabetic Ulcers- National University of Ireland Galway's (WP6) assessed the preclinical safety and efficacy of MSC to the treatment of diabetic ulcers. All three cell types are efficacious in diabetic wound healing but a combination of an *Excellagen* collagen matrix plus the CD362+ cell type demonstrated increased percentage wound healing with increased blood vessel formation.

In **Diabetic Bone fractures- National University of Ireland Galway** (WP7) administration of PA-SSC to a diabetic model of bone fracture has shown these cells are not efficacious in healing fractures. A highly sensitive, quantitative, transferrable biodistribution methodology has been developed, allowing for the detection by quantitative PCR of 1 human cell in 200,000 cells total.
In **Data Management- Pintail Ltd** (WP8) StudyVault, the cloud-based secure data management system has been set up to deal with project specific data and feature requirements.

GMP cell production- Leiden University Medical Center (WP9) includes development the bench-top GMP compliant cell sorter - the MACSQuant Tyto. This device will sort the MSC cells as part of the manufacture of clinical product for administration in the clinical trial.

In **Clinical trial –Steno Diabetes Center, Copenhagen** (WP10) liaison with the DKMA took place and the advisory group at Steno came to the decision to recommend that treatment of diabetic ulcers be taken to clinical trial.

In **Coordination & Administration- National University of Ireland Galway** (WP11) the management proceeded smoothly. Through **Dissemination & Exploitation- Orbsen Therapeutics Ltd** (WP12) the communication of the REDDSTAR project is successful.

Expected final results and potential impact

At present, there are few therapeutic options available to control initiation and progression of diabetic complications and they continue to present challenging disease management issues for clinicians. REDDSTAR has the potential to significantly impact the management and treatment of diabetes, with relevance for clinicians, researchers, pharmaceutical companies, and for the general public. REDDSTAR aims to improve the treatments available for diabetic patients, to enhance their health and quality of life. Ultimately it is hoped that such improvements would lead to a reduction in public health costs associated with diabetes and related complications.

REDDSTAR partners have investigated the safety and efficiency of MSC in resolving the six complications arising from diabetes. The next 18 months of the project will involve examining how MSC improve diabetic complications through mechanism of action studies. REDDSTAR partners are also in the process of preparing a clinical trial application to the DKMA to undertake a phase 1b clinical trial on diabetic patients with diabetic ulcers. The project has generated exciting and novel data and technology that has reach far beyond the life of the project and has the potential to impact on many other disease conditions.

Control of Blood Glucose and Treatment of Complications

There is currently no available treatment that will improve control of blood glucose and simultaneously address underlying diabetic complications. The REDDSTAR Project is novel in its reach across the control of blood glucose and the improvement of a range of serious tissue complications. Initial pre-clinical data has demonstrated direct positive effects of MSC and in particular $CD362^{+}$ SSC on diabetic nephropathy, neuropathy and wound healing with other positive effects on models of oxygen induced retinopathy and effects on immunomodulation in an animal model of diabetic cardiomyopathy. In addition positive effects on blood glucose in combination with the underlying diabetic complication have been demonstrated in an animal model of diabetic nephropathy.

Collaboration

The REDDSTAR consortium is a **network** of diabetes specialists working together with cutting edge regenerative medicine researchers, biotech industrialists and clinicians to develop, validate and translate new breakthroughs in the treatment of diabetes and its associated complications using stem cell therapy. The broad scope of the REDDSTAR project requires unprecedented collaboration between specialists in diverse areas. Common standard operating procedures, data collection and storage mechanisms are enabling valuable comparisons and correlations between samples and results. The consortium has been assembled with the longer-term aim of establishing a durable collaborative scientific network across Europe.

MSC and the MACSQuant Tyto

The REDDSTAR project will involve the use of an antibody (Cyndacel-M) identified by REDDSTAR partners which isolates CD362^{+} SSC, enabling testing of pure CD362^{+} and CD362^{-} SSC and mixed PA-SSC from different species for the first time. Currently available technologies for purifying populations of cells as defined by combinations of antibodies are marginally appropriate for use in therapeutic cell manufacturing. The MACSQuant Tyto employs a closed sterile single-use fluid path which mitigates all of the sample integrity risks associated with droplet sorters, and will be used to manufacture cGMP-compliant clinical doses of MSC which will be administered in the REDDSTAR Phase 1b clinical study.

Our Results

Our results are generating new knowledge in respect of diabetes and related complications. These results have practical application so that this new knowledge can be applied in a clinical setting and will have implications beyond the realm of diabetes and its associated complications.