REDDSTAR: Repair of Diabetic Damage by Stromal Cell Administration (www.reddstar.eu)

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Conflict of Interest

TOB is a founder, Director and equity holder in Orbsen Therapeutics
Diabetes Mellitus

- Global prevalence of DM in 2030 - 439 million adults

- Poor control of blood glucose levels
  - Nephropathy
  - Neuropathy
  - Retinopathy
  - Foot ulcers
  - Cardiomyopathy
  - Impaired bone regeneration
The REDDSTAR Project

- The REDDSTAR Project (www.reddstar.eu), an EU FP7-HEALTH-2012-INNOVATION-1 Grant will examine if MSC/Stromal Stem Cells (SSC) can safely control glycaemia and treat six diabetic complications

- The first 18 months - preclinical safety and efficacy and effect on glycemia

- The second 18 months - MOA studies

- Phase 1b Clinical trial application to the Danish Medicines Agency on the complication(s) that yield the best results in stage 1 of the project
The REDDSTAR Project

- Orbsen Therapeutics has identified a novel antibody (CD362) and can be used to prospectively FACS-isolate CD362+CD45- MSC from human bone marrow with enhanced MSC/MNC purity ratios of up to 1/4.

- Owl Biomedical at Miltenyi Biotec are developing the world’s first bench-top GMP-compliant FACS Nanosorter the MACSQuant Tyto to produce CD362+

- The MACSQuant Tyto will be used to manufacture clinical doses of SSC at the Leiden University Medical Centre (LUMC)

- Cells will be administered in the REDDSTAR Phase 1b clinical study at the Steno Diabetes Centre in Copenhagen
Diabetic Complications AND Glycemia

Clinical Trial

Bone marrow-derived stromal stem cells
Partners

Preclinical Development:

- Prof. Tim O’Brien, NUI Galway: Diabetic Wound/Bone Repair
- Prof. Alan Stitt, Queen’s Belfast: Diabetic Retinopathy
- Prof. H.J. Anders, LMU Munich: Diabetic Nephropathy
- Prof. Isaura Tavares, University of Porto: Diabetic Neuropathy
- Prof. Carsten Tschöpe, Charité, Berlin: Diabetic Cardiomyopathy
- Orbsen Therapeutics: Autoimmune glycaemia – NOD mice

Clinical/GMP Development:

- Prof. Willem Fibbe, Leiden Medical Centre: GMP Manufacturing of clinical-grade MSC
- Owl Biomedical at Miltenyi: GMP FACS Nanosorting of CD362+MSC
- Steno Diabetes Centre and Bispebjerg Hospital: Phase 1b Clinical Trial
Workpackage details

WP1: ORBSEN
Provision of human PASSC, CD362- & CD362+ SSC to other partners and assessment if human SSC can safely reverse STZ-induced hyperglycaemia in STZ-treated NOD/SCID mice

WP2: UPORTO
CD362+, CD362- SSC and PA-SSC from human marrow will be tested in an STZ-induced model of diabetic neuropathy in the Wistar rat

WP3: QUB
CD362+, CD362- SSC and PA-SSC from human marrow will be assessed in two murine models of ischaemic and diabetic retinopathy

WP4: CHT Berlin
CD362+, CD362- SSC and PA-SSC from human marrow will be assessed in two murine models of diabetic cardiomyopathy: the T1 STZ model and a db/db model of T2 diabetes

WP5: MUN
CD362+, CD362- SSC and PA-SSC from human marrow will be assessed in long term murine db/db models of T2 diabetic nephropathy

WP6: NUIG
CD362+, CD362- SSC and PA-SSC from human marrow will be tested in an alloxan induced rabbit model of diabetic wound ulceration

WP 7: NUIG
CD362+, CD362- SSC and PA-SSC from human marrow will be tested in the STZ-induced murine model of impaired bone healing
Workpackage details

WP9: Owl Biomedical at Miltenyi Biotec
Developing the world’s first bench-top GMP-compliant FACS Nanosorter the MACSQuant Tyto

WP10: Steno
Phase 1b Clinical trial with collaboration of Bispebjerg Hospital in Copenhagen

WP9: LUMC
GMP production using MACSQuant Tyto and validation of cell product ready for clinical trial. Shipment of clinical grade product to Copenhagen
Diabetic Foot Ulcers

- 15-25% of people with diabetes will develop foot ulceration

- Diabetic foot ulceration precedes amputation in 80% of cases

- Foot ulceration and amputation negatively impacts patients' quality of life, and incurs substantial financial cost on healthcare budgets

- A high amputation rate exists despite current standards of care
Diabetic Foot Ulcers

Foot Ulcers in Diabetics: Rule of 15

15% diabetic patients one foot ulcer in lifetime
15% of foot ulcers result in amputation

Amputations in Diabetes

• World wide Amputation in T2 diabetes every 30 seconds
• 50% of amputations at transfemoral/transtibial level
• 50% of patients 2nd amputation <5y
• 50% of patients die in <5y
Diabetic Ulcers

Previous preclinical research

- **Hypothesis:** topically applied plastic adherent stromal stem cell (PASSC) therapy augments wound healing in a diabetic wound healing model.

- **Model:** alloxan induced diabetic rabbit ear ulcer model.

- **Methods:** Healthy rabbit derived PASSC were cultured and delivered to a diabetic wound using an ‘in-house’ collagen scaffold. Wound closure and stereology assessed.

- **Results:** The MSC treatment with $1 \times 10^6$ PASSC resulted in increased percentage wound closure compared to untreated wounds and more increased density of blood vessels 1 week post treatment.
Diabetic Ulcers

Previous preclinical research


Topical Administration of Allogeneic Mesenchymal Stromal Cells Seeded in a Collagen Scaffold Augments Wound Healing and Increases Angiogenesis in the Diabetic Rabbit Ulcer

Aonghus O’Loughlin,1 Mangesh Kulkarni,2 Michael Creane,3 Erin E. Vaughan,1 Emma Mooney,1 Georgina Shaw,1 Mary Murphy,1 Peter Dockery,3 Abhay Pandit,2 and Timothy O’Brien1

- The experiments in REDDSTAR are an extension of our initial preclinical work with PASSC
Diabetic Ulcers & REDDSTAR

Aims of preclinical studies:

To compare the efficacy of topical administration of humanCD362+, CD362- and PA-SSC in wound healing in the alloxan-induced rabbit model of diabetic ulceration
**Diabetic Rabbit Ear Ulcer Model**

New Zealand White rabbits (3-3.5 Kg)

Diabetes Induction
Alloxan (150 mg/kg)

Wound creation and application of scaffold + MSCs
CD362+/CD362- /PASSCs

- Experimental week
- Glucose levels
- Weight
- Water Intake
- Behaviour

- Euthanasia
- HbA1c
- Wound Closure
- Histology
Task 1  To compare the efficacy of topical administration of $1 \times 10^6$ CD362+, CD362-, or PA-SSC in wound healing in the alloxan-induced rabbit model of diabetic ulceration

Instead of an ‘in house’ collagen as was used previously, for REDDSTAR we are using Excellagen (Cardium Therapeutics) which is an FDA approved (CE applied for) formulated, high molecular weight fibrillar type I collagen (2.6%) derived from bovine skin. It is homogenous, white in colour, non-cross-linked suspension formulated in a specialized physiologic buffer. Current formulation is: A unit of product is 0.5cc material (2.6% Type I bovine collagen) sterile filled into 1 cc glass syringes for topical application.

Each wound was treated with one of five randomized treatment groups:
1) No treatment
2) Excellagen scaffold alone
3) Excellagen with $1 \times 10^6$ wild type MSCs
4) Excellagen with $1 \times 10^6$ CD362+ type MSCs
5) Excellagen with $1 \times 10^6$ CD362- type MSCs
Results Task 1

Percentage wound closure of cutaneous ulcers 1 week after treatment (n=8)

% Wound Closure

- No Treatment
- Excellagen
- Excellagen+ (CD362- cells)
- Excellagen+ (CD362+ cells)
- Excellagen+ PA-SSCs

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Results Task 1

Masson’s trichrome stain of rabbit ear ulcer wounds

The wounds treated with CD362+ cells mixed with Excellagen showed the most effective wound healing.
Results Task 1

Representative image of neovasculature in Excellagen+ (CD362+) treated wound

In these wounds, formation of new blood vessels (arrows) can be observed within the wound bed.

The significant wound healing which was observed in Excellagen & CD362+ treated wounds by increased percentage wound closure may be associated with more efficient neovasculature.
Results Task 1
Stereological analysis of wounds in diabetic animals

Surface density (Sv) represents the amount of surface area (SA) contained in a reference volume (V). The surface area of a capillary represents the area available for gaseous transport to surrounding tissue. Diabetic wounds treated with Excellagen or a combination of Excellagen & CD362+cells showed significantly increased surface density of blood vessels compared to untreated wounds or other cell types.
Results Task 1
Stereological analysis of wounds in diabetic animals

Length density is a measurement of the length of blood vessel per unit volume of tissue (Lv). Diabetic wounds treated with Excellagen or a combination of Excellagen & CD362+ cells showed significantly increased length density of blood vessels compared to untreated wounds or other cell types.
The radial diffusion distance allows for the measurement of the distance between blood vessels, and is an indicator of the efficiency of a capillary network. The smaller the distance between blood vessels, the shorter distance required for nutrients to diffuse into surrounding tissues. The radial diffusion distance was significantly decreased in diabetic wounds treated with Excellagen or a combination of Excellagen & CD362+ cells when compared to untreated wounds and other cell types.
Conclusions  Diabetic Rabbit Ear ulcer study

Task 1 Topical study

- Study wounds treated with human CD362+ cells mixed with Excellagen showed increased percentage wound closure when compared with untreated wounds or Excellagen treated wounds alone.

- In the present REDDSTAR study, increased blood vessel formation was observed within the wound bed in the Excellagen/CD362+ cell treatment and the excellagen alone groups.

- Beneficial effects
  - Wound closure
  - Surface density
  - Length density
  - Radial diffusion
What’s next for REDDSTAR

- Have now reached mid-point in project and have encouraging preclinical results with CD362+ and Diabetic Nephropathy, Diabetic Neuropathy and Diabetic Ulcers

- Moving on to Clinical development with diabetic complication that determined to be most promising by clinical trial selection committee from Steno.

- GLP toxicology studies and IMPD/IB submitted to Danish regulator

- GMP production of CD362+ by LUMC using OWL/Miltenyi MACS Quant Tyto to release clinical grade batches of cell product

- Phase 1b clinical trial with Danish patients in Steno Diabetes Centre in collaboration with Bispebjerg Hospital
Acknowledgements

REDDSTAR participants

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