MERLIN

MESENCHYMAL STEM CELLS TO REDUCE LIVER INFLAMMATION

WELCOME TO THE WINTER 2017–2018 NEWSLETTER FOR THE EU PROJECT MERLIN

The MERLIN team at a project meeting in Rotterdam in May 2017

CONTENTS

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Refresher Course

A reminder of the project's key aims and objectives

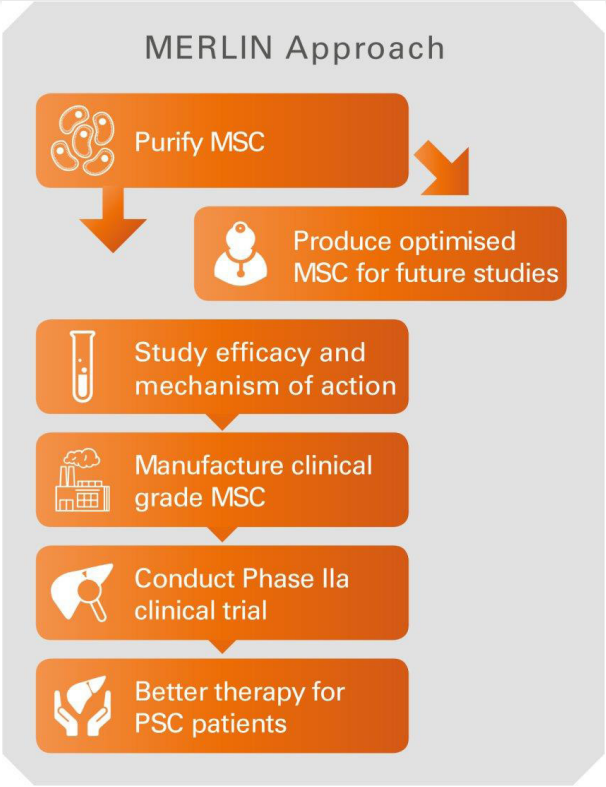
It is estimated that 29 million people in the EU have chronic liver disease, and it is the fifth most common cause of death. Primary sclerosing cholangitis (PSC) is a type of liver disease that is rare and poorly understood. Like most liver diseases, PSC involves inflammation that leads to liver damage. There is currently no curative treatment for PSC, and damage from the disease means that patients often need liver transplants.

MERLIN is exploring new ways to treat liver disease with stem cells from adult bone marrow (MSCs), specifically focusing on PSC as a model disease.

Regenerative medicine uses MSCs to regenerate tissue or organs damaged by disease. However, in order to unlock their full potential, it is necessary to manufacture pure MSC populations under optimum conditions. It is also important to understand more about how MSCs work (mechanism of action), how the body's immune system responds to these cells (immunogenicity) and where MSCs are located in the body after administration (bio-distribution). In Merlin we are studying all these areas.

In the first phase of the project, the MERLIN team have looked at the effectiveness of MSCs against inflammatory liver disease in pre-clinical laboratory models. In the second phase of the project, we are using the results generated to design and implement a clinical trial, looking at the effect of MSC therapy on inflammation in patients with PSC and patients with autoimmune hepatitis. The Merlin clinical trial will set the stage for future work to bring a new, MSC-based therapy to the clinic for these conditions.

MERLIN is specifically focused on PSC and on autoimmune hepatitis, but will also generate new knowledge that is more widely applicable to liver disease, to other conditions involving inflammation and to regenerative medicine in general.



Prof Phil Newsome, University of Birmingham, Coordinator of the Merlin Project

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Forging Ahead

Prof Phil Newsome (University of Birmingham) Coordinator of the Merlin Project: *"The MERLIN programme continues to generate exciting new data about the efficacy and mechanism of action of MSC in the setting of liver injury. We look forward to starting the clinical trial in patients with autoimmune hepatitis and primary sclerosing cholangitis in 2018. Our next meeting is in Padua in 2018 where we will discuss new developments and results."*

Merlin research work and analysis of results is ongoing. However, we have set out a short update on some key work packages below.

Work package 1 in Merlin focuses on testing the efficacy of MSCs in the laboratory. We have made good progress in this WP with exciting new data emerging about the effect of MSCs on endothelial cell activation and amelioration of biliary epithelial senescence. An abstract was submitted to EASL and work continues on a key WP1 publication summarising our results.

Work package 2 explores the functional role of endogenous MSC in pre-clinical and clinical settings of PSCP. This challenging work package is almost concluded with the final experiments ongoing, examining the knock-down of FAP cells in acute CCl4 and MDR2 pre-clinical models. Results will be presented at the Padua meeting.

Work packages 3 and 5 involve research into MSC immunogenicity and enhancement of MSCs respectively. During 2017 researchers at Erasmus Medical Centre published two manuscripts and presented their data concerning the MERLIN project on various occasions at national and international meetings. In addition, two other manuscripts are ready for publication and submission.

Through this work, it was demonstrated that both bone marrow MSC (bmMSC) and umbilical cord derived MSC (ucMSC) have a high proliferative capacity, which is beneficial for the generation of many doses per tissue donor. During expansion both bmMSC and ucMSC remain genetically stable and maintain their immunophenotype. However, the immunosuppressive capacity of the cells was reduced after long-term expansion. Thus, clinical application of a higher passage MSC leads to a higher yield of therapeutic MSC but this may affect the immunotherapeutic efficacy of the cells.

Furthermore, we demonstrated that ucMSC are responsive in a differential manner to various in vitro treatments. As a result of the treatments, MSC modulated their immunophenotype and/or their immunosuppressive capacity. Subsequently, when testing MSC with induced hypo-immunogenicity and enhanced immunomodulatory properties, we observed promising results in an inflammatory liver slice model.

Finally, in the ongoing search to the precise mechanisms of action of MSC, monocytes were identified as key players in the mechanism of action of MSC. Monocytes were observed to phagocytose MSC in vitro as well as in vivo, leading to polarization and relocation. Monocytes that engulfed MSC expressed a more regulatory phenotype, which in vitro led to the induction of FOXp3 cells. This work has recently been accepted for publication in the Stem Cells journal.
The above findings enhance our understanding of the mechanisms of immunomodulatory action of MSC and how to translate this information to the clinic.

Work package 4 addresses the mechanism of action of MSC in models of liver damage in vivo. During 2017 we have identified a novel mechanism used by MSC to modulate angiogenesis. This discovery will pave the way for novel therapeutic interventions. A manuscript detailing our findings will be submitted for publication in the coming months.

Work packages 7 and 8 deal with the production of clinical grade MSC and the implementation of the Merlin clinical trial. Ethics approval for the trial issued in March 2017 and approval from the UK MHRA was received on 3 April 2017. Production of MSCs and trial preparation are well advanced. We look forward to starting the clinical trial in patients with autoimmune hepatitis and primary sclerosing cholangitis in 2018.

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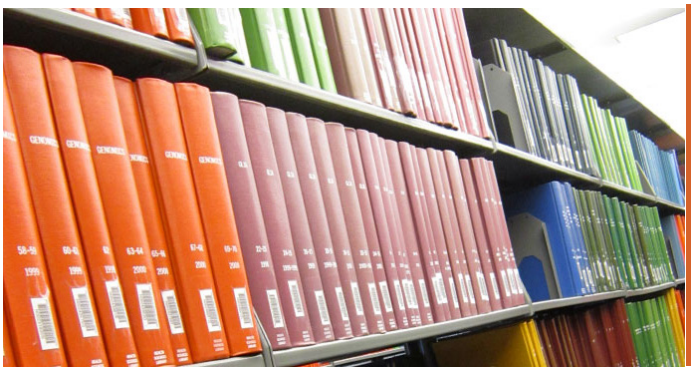
Read all about it!

Details of some recent MERLIN publications



MERLIN research results are still being generated, collated and analysed and we envisage many scientific publications in the months ahead, based on our work. However, the team have already released some significant publications. Recent examples are described below.

"Proteomic analysis of the secretome of human bone marrow-derived mesenchymal stem cells primed by pro-inflammatory cytokines"
(*Elisa Maffioli, Simona Nonnis, Roberta Angioni, Fabiana Santagata, Bianca Calí, Lucia Zanotti, Armando Negri, Antonella Viola, Gabriella Tedeschi*) The Journal of Proteomics, doi.org/10.1016/j.jprot.2017.07.012.

This article addresses the analysis of human bone marrow-MSC primed with pro-inflammatory cytokines. The paper deals with the proteins identified and quantified in stimulated/unstimulated secretome. It also assesses the involvement in inflammation and angiogenesis of proteins enriched in stimulated secretome. The article further concludes that TIMP1 has a key role in anti-angiogenic properties of stimulated secretome.

"Cytokine treatment optimises the immunotherapeutic effects of umbilical cord-derived MSC for treatment of inflammatory liver disease"
(*Samantha F. H. de Witte; Ana M. Merino, Marcella Franquesa, Tanja Strini, Johanna A. A. van Zoggel, Sander S. Korevaar, Franka Luk, Madhu Gargesha, Lisa O'Flynn, Debashish Roy, Steve J. Elliman, Philip N. Newsome, Carla C. Baan and Martin J. Hoogduijn*). Stem Cell Research & Therapy 20178:140 doi.org/10.1186/s13287-017-0590-6.
This paper describes work to improve the immunomodulatory and immunogenic properties of MSC (with the aim of maximising therapeutic effect). The paper outlines findings that treatment of umbilical cord MSCs with multiple cytokines can enhance immunomodulatory capacity and immunogenicity. The results highlight the potential for improving the efficacy of ucMSC as immunotherapy for liver inflammation.

"Aging of bone marrow and umbilical cord–derived mesenchymal stromal cells during expansion"
(*Samantha de Witte, Eleonora E. Lambert, Ana Merino, Tanja Strini, Hannie J.C.W. Douben, Lisa O'Flynn, Steve J. Elliman, Annelies J.E.M.M. de Klein, Philip N. Newsome, Carla C. Baan and Martin J. Hoogduijn*)
Cytotherapy Volume 19, Issue 7, July 2017, Pages 798-807 doi.org/10.1016/j.jcyt.2017.03.071.

Extensive expansion of MSCs is generally required to generate a clinical dose. This piece examines the impact of long-term in vitro expansion on MSC stability and function. The findings show some differences in the efficacy of higher passage MSCs and will help inform future MSC production.

These and other publications related to our work can be found on the MERLIN website at http://fp7merlin.eu/project/the-research-plan/publications-page/

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Out and About

Since the project began, project partners have been involved in numerous dissemination activities. A selection of key presentations delivered, and events attended recently, are summarised below.

Samantha de Witte Erasmus University Medical Centre – lead author on two of the papers. Recent presentations given by Samantha are also referenced here..

The 26th International Congress of The Transplantation Society, Hong Kong: Dr Martin Hoogduijn from Erasmus MC presented at the TTS Conference on 28 August 2016 "Optimizing the immunogenicity and immunomodulatory properties of MSC". Dr Hoogduijn also presented at the **10th UK Mesenchymal Stem Cell Meeting, York, UK** on 5 December 2016. Members of the team from the University of Birmingham were also in attendance.

The 7th European Club for Liver Cell Biology Meeting, Ascot, UK: Dr Ditte Hedegaard from the University of Birmingham presented at this meeting on 6 October 2016 on the "Identification of fibroblast activation protein expressing cells in injured liver".

Meeting of The Transplantation Society in Victoria, Canada: Dr Martin Hoogduijn from Erasmus MC presented at this meeting on 24-26th May 2017. The presentation was focused on MSC biology.

EU-MSC2 meeting, Leiden, NL: Hosted by Leiden University Medical Center, the EU-MSC2 2017 meeting in Leiden, NL on September 12th and 13th assembled twelve EU-funded mesenchymal stem cell-focussed consortia. MERLIN was represented by Dr Steve Elliman, CSO and Dr Lisa O' Flynn, Head of Process Development at Orbsen Therapeutics; Danielle Nicholson, Pintail Limited, and Dr Jon Smyth, NHS Blood and Transplant. At this two-day, interactive meeting the themes explored included: MSC mechanisms of action and potency assays; product development and market authorisation in a changing regulatory landscape; progressing clinical translation of MSC research and overcoming hurdles in clinical trials with MSC therapies.

The interactive meeting provided opportunities to enhance knowledge-sharing between EU research groups working in the MSC biology domain; engage with European Commission Project Officers, stem cell ethicists, the European Medicines Agency (EMA) and other stakeholders; assemble trans-disciplinary research groups;

MERLIN at EU-MSC2. Orbsen Therapeutics's Dr Steve Elliman presents on related projects (L) and MERLIN colleagues at the meeting (R)

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Out and About (contd)

introduce early stage researchers to new networks and explore future consortium building and international funding opportunities.
The meeting will serve collectively to enhance the quality and impact of planned clinical trials and progress the clinical translation of MSC research and developments.

The European Society for Organ Transplantation (ESOT) Congress, Barcelona, Spain: Samantha de Witte (Erasmus MC) presented "Impact of culture expansion and inflammatory cytokine challenge on DNA methylation profiles in MSC" at the ESOT Congress on 25 September 2017. Franka Luk (Erasmus MC) also presented "Tracking and fate of umbilical cord-derived MSC after intravenous infusion in mice".

European Haematology Association (EHA), SWG Scientific Meeting on Shaping the Future of Mesenchymal Stromal Cells Therapy, Amsterdam, NL: on 23 November 2017 Samantha de Witte presented an abstract entitled "Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells".

These presentations are just a selection of the events and meetings at which the Merlin team presented over recent months. We are looking forward to many more such presentations in the months ahead.

Plenary Meeting

The Merlin team met in Rotterdam for a very successful plenary on 10 and 11 May 2017. Updates were presented on each WP. MSC production and preparation for the upcoming trial were a key focus. Publications and plans for future work were also discussed. Many thanks to EMC for hosting. The partners look forward to the next plenary hosted by UNIPD in Padua on 22 and 23 January 2018!

Members of the MERLIN team at the plenary meeting in Rotterdam, 10 and 11 May 2017.

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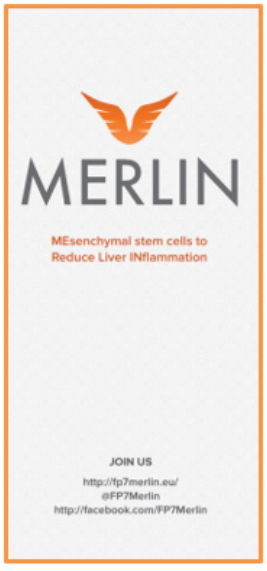
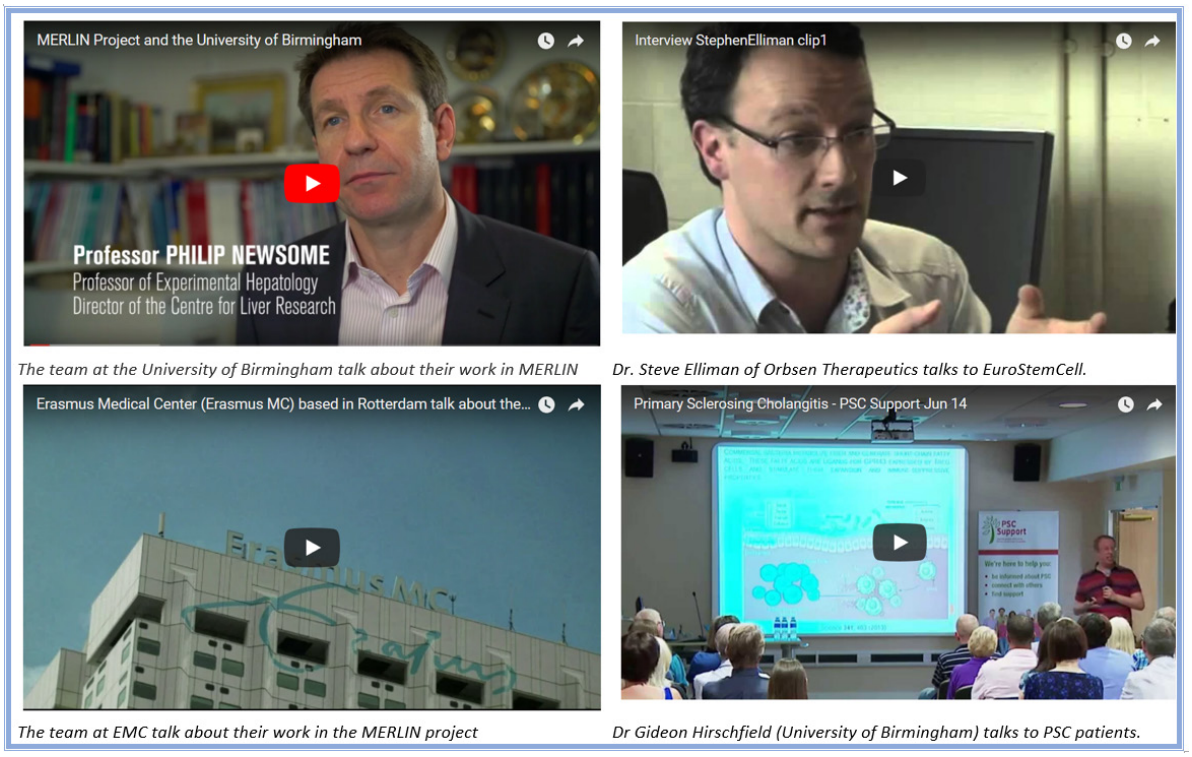
MERLIN Materials

Over the course of the project we have produced various materials which you can access to find out more about our work. These materials are all available from the Project Website.

See our Project Flyers, Newsletters, Progress Reports and videos (as below).





The team at the University of Birmingham talk about their work in MERLIN

Dr. Steve Elliman of Orbsen Therapeutics talks to EuroStemCell.

The team at EMC talk about their work in the MERLIN project

Dr Gideon Hirschfield (University of Birmingham) talks to PSC patients.

http://fp7merlin.eu/media-centre/photos-videos-and-presentations/

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PROJECT PARTNERS

MERLIN brings academic and clinical experts in stromal cell biology, inflammation and liver disease together with industry leaders in stromal cell manufacturing and advanced imaging, to develop a therapy for patients with PSC. The partners in the project are set out below. For more details see http://fp7merlin.eu/partners/

University of Birmingham, United Kingdom

NHS Blood & Transplant, United Kingdom

Orbsen Therapeutics Limited, Ireland

Università Degli Studi Di Padova, Italy

Erasmus Medical Centre, Netherlands

BioInVision, United States

Pintail Ltd, Ireland

For further information on the project please see the Project website http://fp7merlin.eu/. Join the conversation!
You can also follow us on Twitter (https://twitter.com/fp7merlin) or Facebook (https://www.facebook.com/FP7MERLIN/).

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