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FINAL REPORT

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Name, title and organisation of the scientific representative of the project's coordinator:
Prof. Philip Newsome
Head of Cell Therapy, Director of the Centre for Liver Research
School of Immunity and Infection, University of Birmingham
Tel: +44 (0)121 415 8700
Fax: N/A
E-mail: p.n.newsome@bham.ac.uk
Project website address: www.fp7merlin.eu

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1. Final publishable summary report

1.1 *Executive summary*

It is estimated that 29 million people in the EU have chronic liver disease, and it is the fifth most common cause of death. Most liver diseases involve inflammation that leads to liver damage. MERLIN is focused on developing a stromal cell therapy for the liver diseases primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH). There are currently limited treatment options available for these conditions.

Researchers in MERLIN have looked at the effectiveness of Mesenchymal stromal cells (MSCs) against inflammatory liver disease in the laboratory, in order to inform and underpin a novel cell therapy. We have also explored mechanisms of action of MSCs and optimum conditions for MSC production.

Some of our key findings include:

- MSCs reduce markers of liver damage and inflammation in laboratory models of inflammatory liver disease, as well as the number of inflammatory cells in damaged areas.
- MSCs derived from both *bone marrow* (BM) and *umbilical cord* (UC) show positive effects.
- The route of infusion of UC MSC has no impact on its therapeutic effect (MSCs infused subcutaneously and intravenously were equally effective).
- The beneficial effect of MSCs on PSC is due, at least in part, to the extracellular vesicles (EVs) that MSCs secrete.
- The properties of MSCs can be influenced by the conditions under which the MSCs are cultured. Culturing protocols also merit due consideration.
- MSCs largely move to the lungs following administration, with an accumulation of dead MSCs in the liver 24h after infusion.

In MERLIN we used the results of our studies to design a clinical trial to test the safety of our MSC therapy and examine the effect of therapy on inflammation in patients with PSC and AIH. We also developed a process for MSC production from UC tissue that is consistent and aligned with regulatory requirements. NHSBT have manufactured specially selected MSCs (ORBCEL-C™, discovered by Orbsen Therapeutics) for delivery to patients in the trial. The MERLIN trial opened on 7 December 2018 and will continue (and be completed) after the project ends.

We believe that our work will set the stage for a new, MSC-based therapy for PSC and AIH, with potential application to other forms of liver disease and other inflammatory conditions. The project has delivered important data and lays the groundwork for the future tailored production and use of precisely characterised MSCs for particular applications.

In addition to potential benefits for individual patients and their families, improving treatments for liver disease will also benefit healthcare systems, through the reduction of costly liver transplant procedures and of care costs associated with chronic liver disease. From a health economics perspective, MSC therapies for immune and inflammatory diseases offer potential for significant savings in the longer term. Researchers in liver disease, immunology and regenerative medicine will benefit from the new knowledge generated by the project and the MERLIN trial. An additional benefit derived from the project will be the advancement of 3D imaging technology, which offers researchers new, better ways to carry out bio-distribution studies.

The MERLIN consortium is committed to building on the project in future collaborations, generating long-term research value and delivering societal and economic benefits through the development of advanced MSC therapies.

1.2 *A summary description of project context and objectives*

The overall objective of MERLIN was to develop and validate (in a Phase 2a clinical trial) purified MSC as an entirely new treatment option for patients with inflammatory liver diseases PSC and AIH, in parallel with enhancing knowledge about the operation of MSCs.

Background

The prevalence of chronic liver disease has been estimated to be 6% (or 29 million people) in the EU (*The European Liver Patients Association. 2005*), with mortality rates estimated at 14.3 per 100.000 in the EU-25 in 2004, making it the 5th commonest cause of death (*the EU Statistical Year Book, 2007*). Most liver diseases have a significant inflammatory component that underpins liver damage & fibrogenesis, yet current therapies have limited effectiveness. Primary Sclerosing Cholangitis (PSC) is an example of chronic immune mediated liver injury, with a natural history of progression to liver failure. The incidence and prevalence rates for PSC vary from 0 to 1.3 per 100,000 inhabitants/year and 0 to 16.2 per 100,000 inhabitants, respectively. Liver transplant is ultimately the only viable treatment for many people with PSC.

Our challenge in MERLIN was to develop a therapy that alleviated the ongoing inflammation that drives fibrosis in liver injury. Using PSC and AIH as our paradigm reflected the inflammatory and immune mediated nature of these conditions, alongside the huge unmet need for therapy. We aimed to harness laboratory and clinical studies to effectively, and in a timely manner, answer our questions regarding optimised MSC therapy and how such therapy might be applied in the context of PSC and AIH.

MERLIN Key Objectives

As noted above, the main objective in MERLIN was to develop a Phase 2a clinical trial administering purified MSC as an entirely new treatment for PSC and AIH patients, in tandem with developing new knowledge about MSCs.

Some more specific and detailed objectives of the project described in the original programme of work are set out below.

Translational Aims (related to the MERLIN clinical trial)

The aims below were devised at the outset of the project to help us deliver the proposed clinical trial.

- *To compare the safety and efficacy of ORB1+ and ORB1- MSC to PA-MSC in refined models of PSC* – this aim was designed to help us select the most appropriate MSC-based product for use in the MERLIN trial.
- *To ensure EU ATMP compliance and prepare an application for a Phase 2a Clinical Trial of ORB1 therapy in PSC* – this aim involved the generation of an Investigational Medicinal Product Dossier (IMPD) Investigator Brochure (IB) and Clinical Trial Protocol for the MERLIN Clinical Trial Application to the Medicines Health and Regulatory Authority (MHRA) in the UK.
- *To carry out a Phase 2a Clinical Trial of ORB1 therapy in PSC* – this aim related to the validation of the selected MSC-based therapy in the MERLIN clinical trial.
- *To establish the in vivo immunological footprint of MSC after infusion into pre-clinical models of PSC and also in clinical trial patients* – this aim set out practical tests to be applied in order assess the impact of the therapy administered.

Development Aims (increasing knowledge about MSCs)

The following aims were identified at the start of the project in order to guide our studies into MSCs and help us develop a deeper understanding of MSC operation.

- *To elucidate the mechanism of action of MSC in ameliorating liver inflammation* -this aim required us to explore the role and mechanisms of action (MoA) by which MSC simultaneously alleviate liver inflammation and repair tissue damage.

- *To improve the functional longevity of MSC* – this aim required an assessment of culture protocols for MSCs, to determine at what point in the culture process their efficacy may be compromised.
- *To reduce the immunogenicity of MSC* – this aim focused on exploring how to modulate the potential susceptibility of MSC to immune cell recognition, in an effort to improve survival of MSCs post infusion.
- *To improve the homing/localisation of MSC* – this aim required us to consider the homing and biodistribution of MSCs post infusion and the most effective ways to administer MSCs.
- *To generate the optimal MSC* – this aim required the study of conditions which might optimise the operation of MSCs.

Legacy Aims (To maximise the long-term research and collaboration value of MERLIN).

Specific aims in relation to MERLIN's long-term legacy were clearly articulated in the MERLIN programme of work:

- *To agree a Memorandum of Understanding between the partners to guide long term collaboration with potential to expand and add new members.*
- *To agree standard operating protocols for isolation, culture, storage, transport and use of MSC.*
- *To publish training and best practice materials for the wider research community.*
- *To develop training opportunities across the consortium***.**

Conclusion

We put the aims and objectives outlined above into practical application as we embarked on the MERLIN work programme. We explored the Development Aims described and discovered new knowledge about MSCs. MERLIN partner BIO succeeded in further developing their 3D imaging technology (CryoViz™) for high-throughput imaging and analysis. Different elements of our research emerged as more or less important as our studies generated novel results and directed our focus.

We applied the new information learned in the design of the MERLIN clinical trial, which is now underway. NHSBT have manufactured specially selected MSCs (ORBCEL-C™, discovered by Orbsen Therapeutics) for delivery to patients in the trial.

Details of the scientific work undertaken and key results achieved are set out in section 1.3 below.

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

Introduction

Mesenchymal stromal cells (MSCs) represent a promising therapeutic approach in many diseases, including inflammatory liver disease, in view of their potent immunomodulatory properties. Researchers in MERLIN have looked at the effectiveness of MSCs against inflammatory liver disease in laboratory models, in order to help develop a novel cell therapy for PSC and AIH. A clinical trial was designed based on the outcomes of our studies in the laboratory. The trial is now underway, looking at the safety of the MSC therapy and examining the effect of therapy on inflammation in patients with PSC and AIH. NHSBT have manufactured specially selected MSCs (ORBCEL-C™, discovered by Orbsen Therapeutics) for delivery to patients in the trial. This will set the stage for a potential new, MSC-based therapy in the future. In addition, MERLIN is generating new knowledge that is more widely applicable to MSC therapy in general, informing the development of therapies for other forms of liver disease and other inflammatory conditions.

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

- WP1 Pre-clinical efficacy testing of MSC.
- WP2 Functionality of endogenous MSC.
- WP3 Immunogenicity and immunological footprint of MSC in PSC.
- WP4 MoA of MSC in models of liver damage in vivo.
- WP5 Genetic, biological & pharmacological enhancement of human ORB1+MSC re liver inflammation treatment.
- WP6 Advanced tools for bio-distribution of MSC.
- WP7 Generation of Investigational Medicinal Product Dossier and Manufacture of Clinical Grade ORB1+/- MS
- WP8 Phase 2a clinical trial of ORB1+/- MSC in patients with PSC

In addition, the data management and samples management work carried out in WP9, to support the S&T core, are briefly described.

1.3.1 WP1 Pre-clinical efficacy testing of MSC

1.3.1.1 WP1 Introduction

Encouraging results obtained from pre-clinical studies have highlighted the immunomodulatory and clinical potential of mesenchymal stromal cells (MSCs) Consequently, many autologous and allogenic MSC based clinical trials are being undertaken for a number of indications including graft versus host disease (GVHD) and acute and chronic liver disorders. Nevertheless, transplantation of third party MSCs has been shown to be feasible and safe with good tolerability profiles in clinical studies. There are still uncertainties about their long-term therapeutic efficacy. Although some clinical studies have undoubtedly demonstrated clinical efficacy of MSC, most of the studies often yield mixed results, suggesting a significant variability in the immunomodulatory potency of MSC. It is now accepted that the therapeutic potential of MSCs mainly relies on their short-term paracrine ability to reduce inflammation, inhibit immune responses, and produce trophic factors. An important requirement for the clinical usage of MSC would be the production of clinical grade cells with better treatment efficiency. Therefore, in this reporting period in WP1 we set out to investigate whether pre-treatment of ORB^+ UC-MSC with either IFN-γ+TGF-β +retinoic acid (RA) or the glucocorticoid steroid budesonide would enhance their immunomodulatory and therapeutic efficacy in the Mdr2Ko/FVB pre-clinical murine model of inflammation mediated liver injury. WP3 and WP5 have previously demonstrated that priming of ORB^+ UC-MSC with EMC-cocktail or budesonide enhanced their immunomodulatory potential *in vitro*. Also, their mechanism of action will be elucidated using mono- and co-culture of MSC with hepatic sinusoidal endothelial cells (HSEC) and biliary epithelial cells (BEC) *in vitro.*

1.3.1.2 WP1 Key Work Undertaken and Results Achieved

The overall objective of MERLIN is to bring optimised second-generation MSC therapy to the clinic to treat patients with Primary Sclerosing Cholangitis (PSC).

Cellular senescence of hepatocytes, cholangiocytes, stellate cells and immune cells has been implicated in chronic liver disease progression. We have showed that hydrogen oxide can induce senescence (growth arrest) in human biliary epithelial cells. Furthermore, treatment with MSC or MSC conditioned medium reduced biliary epithelial cell senescence adding further to their beneficial effects. In addition, MSC suppressed the proliferation/activation of injurious inflammatory cells (lymphocytes) isolated from both the peripheral blood of patients with PSC and the explanted liver of patients with PSC who have undergone liver transplant. These data confirm the beneficial effects of ORB+ MSC on human effectors of inflammatory liver disease.

Our pre-clinical studies demonstrated that ORB+ MSC from bone marrow and umbilical cord attenuated hepatic inflammation in Mdr2KO/FVB models of biliary injury. Notably, MSC infusion improved liver injury as evidenced by a reduction in serum alanine aminotransferase (ALT) and bile acid (BA) levels, hepatic pro-inflammatory immune infiltrates and the generation of T regulatory cells and anti-inflammatory macrophages. We demonstrated this effect held true for other models of liver injury including Ova-Bil and CCl_4.

Our *in vitro* data demonstrated that MSC could exert their inhibitory effects locally and/or remotely to down regulate leukocyte recruitment. We also described the involvement of IDO in this phenomenon since blocking this enzyme with 1-MT removed the inhibitory effect of not only MSC but also conditioned medium collected from unstimulated/stimulated MSC. This could in turn reduce the expression of VCAM-1 and ICAM-1. Building on this work we were able to demonstrate that MSC could exert similar beneficial effects *in vivo* after subcutaneous administration suggesting their ability to exert their effects remotely without a requirement to home to the liver.

We sought to establish if we could further improve the effectiveness of MSC by pre-treating them with cytokines. Having tried multiple cytokine combinations we were able to see changes in gene expression but no major differences in terms of their efficacy in vivo in models of liver injury. Therefore, this study suggests that good clinical grade unstimulated ORB^+ UC-MSC may substantially contribute to promising clinical outcomes in an appropriate physiological pro-inflammatory micro-environment without any pre-treatments.

1.3.1.3 WP1 Conclusion

This work package has been successful in demonstrating the effectiveness of MSC in reducing liver injury in a number of different models as well as providing evidence of efficacy with human cells. It also provides insights as to the mechanism of action which will be useful in future studies.

1.3.2 WP2 Functionality of endogenous MSC.

1.3.2.1 WP2 Introduction

This work package, WP2, aims to elucidate the functionality of endogenous MSCs during liver injury by significantly depleting their numbers and subsequently monitoring the development of liver injury in specific mouse models. In 2010, Kraman and colleagues developed a conditional knock-out murine model, which allows the depletion of stromal cells, including bone marrow MSCs. These transgenic mice are biologically engineered to express the human diphtheria toxin receptor (DTR) and Luciferase (Luc)2 in cells which naturally express fibroblast activation protein (FAP), a surface protein marker identified on a range of stromal cells, including human and mouse MSCs (Bae et al., 2008; Tran et al., 2013). Administration of diphtheria toxin (DTx) to these mice results in the depletion of any cell expressing FAP via the engagement of the DTR protein. This FAP-DTR murine model should allow us to determine the impact of stromal cell depletion, including populations of MSCs, during liver injury. A limitation of this model is that a range of other non-MSC FAP+ stromal cell populations, identified in muscle, pancreas and lymph node tissues, are also targeted by the DTx treatment, making it difficult to directly attribute any effects observed to the depletion of MSCs. Nevertheless, this initial study could potentially inform future more in depth studies.

1.3.2.2 WP2 Key Work Undertaken and Results Achieved

We have demonstrated that the expression of the FAP protein was significantly increased at the gene and protein level in chronically diseased human liver tissue when compared to normal liver, as confirmed via qPCR and western blotting. Also, we showed the tissue distribution of FAP via immunohistochemical staining is largely restricted to portal areas and fibrotic septa in chronically diseased tissues. Furthermore, we showed that FAP protein expression strongly correlated with the extent of fibrosis in the diseased tissues, as measured by picrosirius red analysis. This was also confirmed at the mRNA level, with FAP mRNA expression exhibiting a strong positive correlation with collagen mRNA expression (Coll IAI). Additionally, in the previous periods, we started to characterise the expression of FAP in murine liver tissues, with initial experiments demonstrating an increase in FAP mRNA expression in the biliary injury model, MDR2-/-, but no significant changes were observed in acute and chronic carbon tetrachloride (CCl4) models of injury.

Finally, we were able to optimise the murine model which utilised diphtheria toxin (DTx)-mediated depletion of FAP+ stromal cells followed by acute CCl4-driven liver injury. However, it became apparent that the depletion of FAP+ stromal cells significantly affected a number of tissues due to the wide expression of FAP in tissues, such as muscle, pancreas and lymph nodes, making it difficult to directly attribute any effects observed to the depletion of MSCs.

Nevertheless, our results suggest that endogenous populations of stromal cells are not simply bystanders during the administration of exogenous MSCs and could potentially play a role in the pathology of both acute and chronic liver injuries. However, it is unclear from the current data whether that role is beneficial or in fact detrimental. Additionally, these data suggest that the effects of depletion are very much dependent on the context of injury type (acute or chronic) and stage (active injury or resolution) and it is evident that further investigation, beyond the scope of MERLIN, is warranted.

1.3.2.3 WP2 Conclusion

We have comprehensively characterised the expression of FAP in murine and human liver injury which will be of significant value to the scientific community. In so doing it has become clear that FAP expression is not limited to just MSC and thus interpretation of knock-down of FAP is challenging. Our data set the scene for further studies that will need to refine this model further if the effect of MSC depletion alone is to be understood.

1.3.3 WP3 Immunogenicity and immunological footprint of MSC in PSC.

1.3.3.1 WP3 Introduction

WP3 developed around 3 key questions concerned with optimisation, mechanisms of action and monitoring of MSC treatment for immunoregulatory therapy. The first question was whether pre-treatment of MSC *in vitro* could optimise their immunoregulatory efficacy and reduce their immunogenicity. This question was addressed by treating MSC with a variety of factors and determining the effect on MSC immunophenotype, gene expression profile, immunoregulatory function and susceptibility for cytotoxic lysis. We furthermore examined the effect of prolonged culture on MSC phenotype and function and we performed whole genome methylation profiling to examine whether culture-induced changes would leave an epigenetic imprint in MSC.

The second question in WP3 was to elucidate the mechanism of action of MSC therapy. While it was previously thought that the effect of MSC is dependent on the persistence of MSC after infusion, we and others determined that MSC are short lived after infusion. This raises the question of how MSC modulate the immune system after infusion, which we addressed by tracking experiments and measurement of immune parameters after MSC infusion. We also examined whether MSC with optimised immune properties would show a different biodistribution and survival pattern after infusion.

The third question was whether MSC infusion would leave an immunological footprint in PSC patients. To examine this we performed in depth analysis of the immunological footprint in different groups of inflammatory liver disease patients and compared them to healthy controls. We determined immune cell subset distribution and performed serum analyses. These experiments served to setup methodology for measurements of samples from the Merlin clinical trial and provided a background pattern of immune parameters in PSC patients.

1.3.3.2 WP3 Key Work Undertaken and Results Achieved

Experiments were carried out *in vitro* to treat umbilical cord-derived MSC (ucMSC) with a variety of cytokines, growth factors, vitamins and prostaglandins and examine their immunophenotype, gene expression profile and functionality in T cell suppression assays. Of all tested factors, IFNg was the most potent factor in upregulating the expression of key molecules in MSC involved in their immunoregulatory effect, including IDO and PD-L1, and in enhancing the capacity of MSC to suppress T cell proliferation and IFNg production. At the same time, IFNg containing pre-treatments increased the expression of HLA class I and II molecules on MSC, impacting the susceptibility of MSC for cytotoxic attack. To examine whether pre-treatment would modify the epigenome of MSC, whole genome methylation analysis was performed. Only minor effects on the DNA methylation profile of MSC were detected, indicating that the phenotypical changes induced by pre-treatment were transient. Pre-treated MSC were administered via tail vein injection in a CCl_4 inflammatory liver disease mouse model. The differentially treated MSC showed no differences in biodistribution and accumulated in the lungs. There was also no difference in MSC survival and there was no therapeutic effect of control MSC or pre-treated MSC in this model in our hands. Possibly the lack of MSC distribution to the liver prevented a potent effect of MSC on liver inflammation. Therefore we set up an *ex vivo* liver tissue slice model. In this model MSC treated with a combination of IFNg, TGFb and retinoid acid were most potent in reducing the expression of inflammatory factors after challenging the tissue with LPS (Figure 1).

In addition to treating MSC with growth factors and cytokines, we examined the effect of prolonged culture on the phenotype and function of MSC. Culturing MSC higher passage numbers (from 4-12) reduced their proliferation and their capacity to inhibit T cell proliferation. It was also observed that within a passage MSC showed large differences in DNA methylation patterns from time of seeding until confluency. This was most likely associated with cell density.

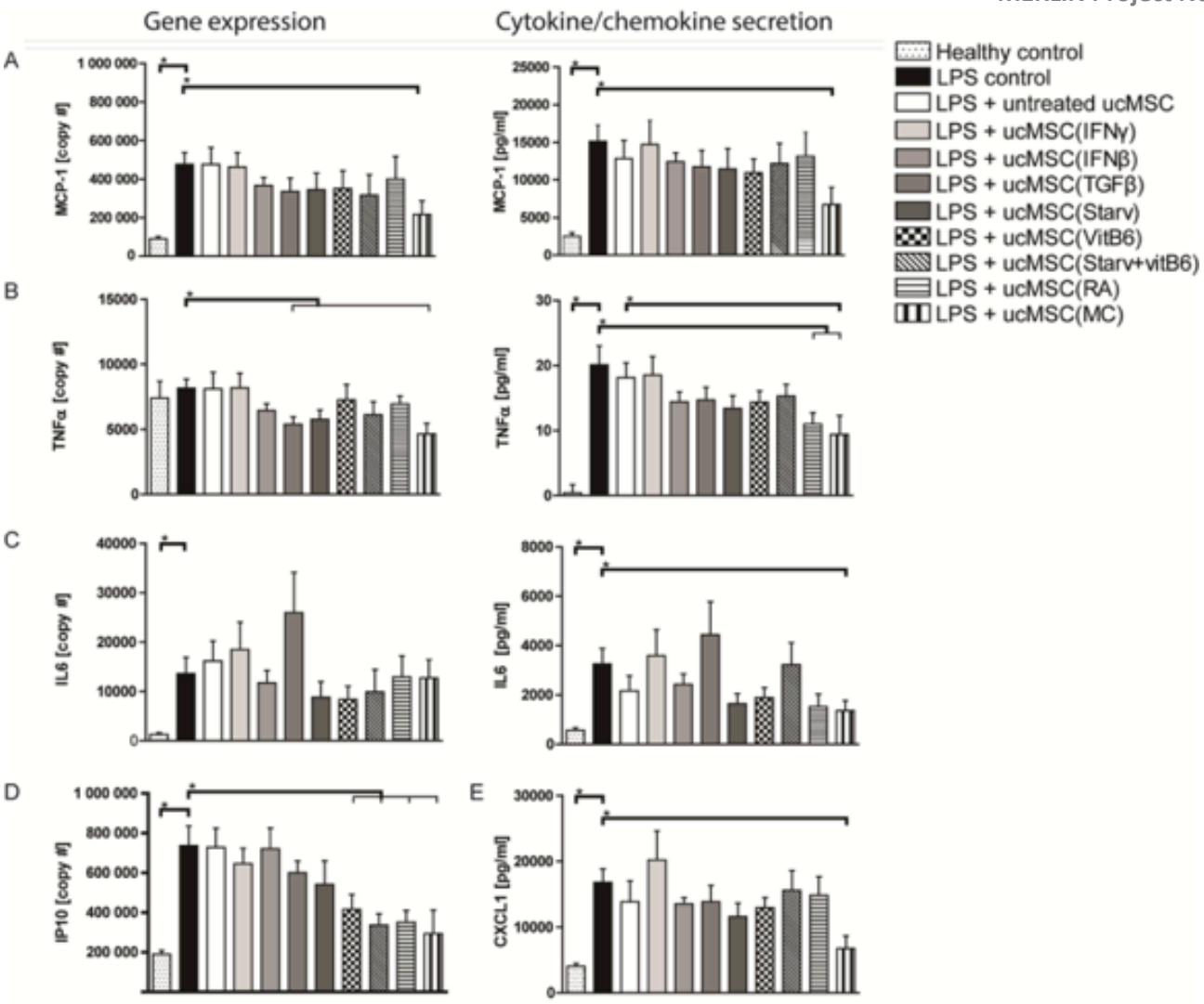


*Figure 1 Effect of differentially pre-treated ucMSC on ex vivo liver slices. Gene expression levels and cytokine/chemokine levels were measured in liver slices treated with LPS and supernatant, respectively. (a)(b)(c) Both gene expression levels and cytokine secretion levels were measured for MCP-1, TNFα and IL6. In addition, (d) gene expression levels of IP10 and also (e) CXCL1 was measured in the medium were measured. n=6 * p<0.05.*

The mechanisms of action of MSC therapy were explored by examining the fate of infused MSC. As explained above, intravenously infused MSC accumulate in the lungs. Double labeling of MSC with Qtracker605 beads, which would disappear from the cells after cell death, and Hoechst, which would be preserved even after cell death, allowed tracking of infused MSC even after death. It was found that directly after infusion the majority of the cells is alive and present in the lungs. After 24h most cells have died and translocate partly to the liver. After 72h no living or dead MSC were detected anymore (Figure 2). The presence of MSC in the lungs was accompanied with elevated numbers of monocytes and neutrophils in lung tissue, which phagocytosed the administered MSC. Monocytes that phagocytosed MSC were subsequently detected in the blood and in liver tissue. Upon phagocytosis of MSC, monocytes adapted a regulatory phenotype and adapted the capacity to induce regulatory T cells. .

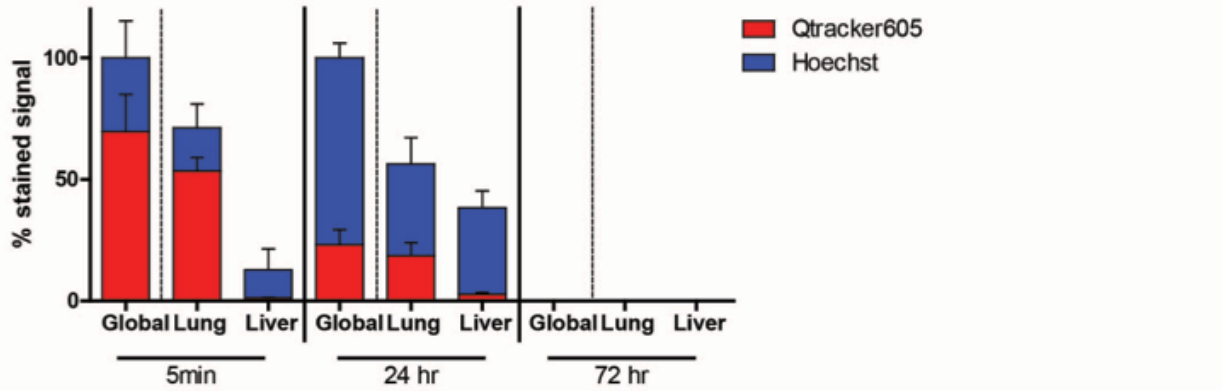


Figure 2 Quantitative detection of double-labeled MSC after intravenous infusion in whole mice, and specifically in lungs and liver. The Qtracker signal detects living MSC, whereas the Hoechst signal also detects dead MSC.

To examine the immunological footprint of PSC patients and other inflammatory liver disease patients, blood samples were analysed for immune cell subset frequencies, profiles of plasma cytokines and growth factors and concentration and size of nanoparticles in plasma. We determined naïve, central memory, effector memory and late effector memory CD4 and CD8 T cell frequencies, B cells, NK cells and different types of monocytes and dendritic cells. Distinct immune cell profiles were observed for different groups of inflammatory liver disease patients. PSC patients for instance showed increased percentages of $CD19^+$ B cells compared to other inflammatory liver disease patients and healthy controls (Figure 3). Furthermore, on the basis of plasma cytokine and growth factor profiles and nanoparticle levels distinctions between different patient groups and healthy controls could be made. The deviated immunological footprint of PSC patients and other inflammatory liver disease patients compared to healthy controls indicates that immune profiling of patients in MSC trials may reveal efficacy and safety information.

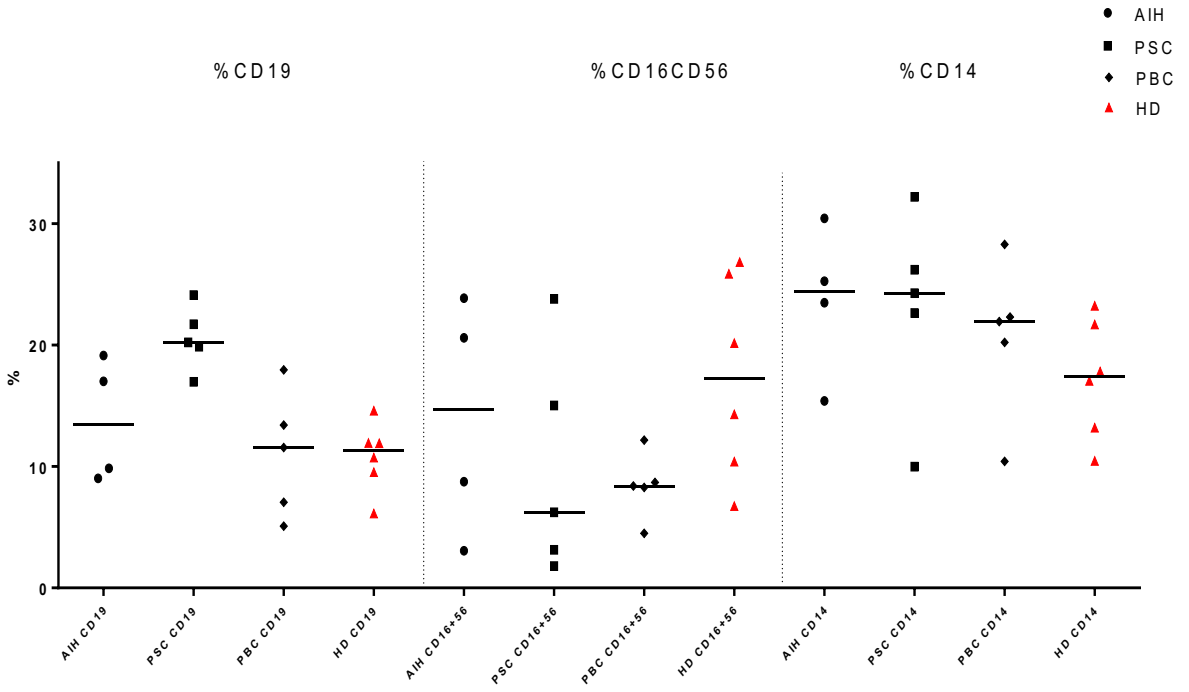


Figure 3 Frequencies of $CD19^+$ B cells, $CD16^+CD56^+$ NK cells and $CD14^+$ monocytes in liver disease patients and healthy donors.

1.3.3.3 WP3 Conclusion

WP3 revealed novel mechanisms of action of MSC therapy. MSC end up in lung tissue after infusion, where they interact with innate immune cells that subsequently adapt a regulatory phenotype and migrate to other sites of the body. Methodology was set up and tested to track MSC after administration and to determine effects of MSC therapy. The immunological footprint of inflammatory liver disease patients was determined, which will serve as a baseline for the Merlin clinical trial.

1.3.4 WP4 MoA of MSC in models of liver damage in vivo.

1.3.4.1 WP4 Introduction

The overall goal of MERLIN is to develop and validate a new treatment option for patients with Primary Sclerosing Cholangitis (PSC) and enhance the functionality of Mesenchymal Stromal Cells (MSC). MSC represent a promising therapeutic approach in many diseases, including inflammatory liver disease, thanks to their potent immunomodulatory properties . MSC inhibit in fact T cell activation induced by an anti-CD3/CD28 antibody stimulus, mitogens, and alloantigens; they also inhibit NK cell activation, as well as B cell terminal differentiation, and dendritic cell maturation and functionality. However the molecular mechanisms responsible for the anti-inflammatory effects of MSC in liver disease are still unknown. Much of the pre-clinical work in liver injury showed that MSC reduce oxidative stress and cellular infiltrate in the liver but it has not been yet defined the mechanism by which this was achieved.

The specific objectives of WP4 were:
1. to identify the environment/locational requirements for MSC-mediated immunomodulation in vivo
2. to identify the molecule/s responsible for the anti-inflammatory effects of MSC in vivo
3. to identify the action of MSC on the hepatic vasculature in suppressing inflammation

1.3.4.2 WP4 Key Work Undertaken and Results Achieved

In order to identify the environment/locational requirements for MSC-mediated immunomodulation in vivo we exploited MSC encapsulation that provides conclusive data in support of either the endocrine or the paracrine/contact signalling required for MSC immunomodulation . Thus, we injected alginate-encapsulated human CD362+ MSCs (E-MSCs) in a mouse model of local inflammation induced by s.c. injection of CFA/OVA . Our results clearly showed that BM CD362+MSCs exert their immunomodulatory properties in vivo, even better than unsorted MSCs, but encapsulation may be detrimental in the case of human, sorted MSCs. (Figure 4)

To formally demonstrate that s.c. injected WT or CD362+MSCs do not leave the injection site, in collaboration with Bioinvision, we performed whole-mouse cryo-imaging analysis of mice immunized with CFA/OVA and injected with fluorescently labelled hMSCs, confirming that neither WT nor CD362+MSCs migrated away from the site of injection and are able to dampen inflammation through the release of soluble mediators.

In an effort to understand the molecular mechanisms responsible for the endocrine effects of MSC, we exploited a standardised workflows previously developed in our laboratories for shotgun proteomic characterization of the mouse MSC secretome . Our results on MSC secretome indicated that, upon activation by inflammatory cytokines, MSC upregulate the expression of several proteins potentially affecting angiogenesis and inflammation through multiple pathways. Interestingly, when we compared the secretomes of human and mouse MSCs, we found that only 16 proteins are upregulated in both cell types and 11 of them modulate angiogenesis directly or indirectly, thus supporting the idea that the endothelium is a specific target of MSC during inflammation. Among the molecules upregulated upon cytokine stimulation, we focused our attention on the tissue inhibitors of metalloproteinases 1 (TIMP1). Thus, we assessed the role of TIMP-1 secreted by MSC in the CFA/OVA mouse model of local inflammation, demonstrating that MSC inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1 and further identifying the endothelium as a specific and novel target of MSC-based therapy. (Figure 5**).** Therefore, we proposed that pre-activation with pro-inflammatory cytokines strengthened the anti-angiogenic effects of MSC, thus supporting our hypothesis that, during an inflammatory response, MSC target angiogenesis and thus dampen the inflammatory response. Additionally, in agreement with data obtained with mouse MSCs, soluble factors secreted by human MSCs affected the tubulogenesis of HUVEC cells . However, in the case of human cells, MSC-conditioned media (CM) was able to inhibit tube formation even when MSC had not been primed by cytokines. Of note, in the case of human MSC, TIMP-1 blockade restored the formation of the HUVEC endothelial network in the presence of either unstimulated (unstim) or stimulated (stim) human MSC-CM, thus pinpointing TIMP-1 as a crucial MSC-derived modulator of angiogenesis also in human models. (Figure 6)

To investigate the role of MSC in modulating angiogenesis in inflamed livers, we performed tube formation assays of human sinusoidal endothelial cells (HSECs) from PSC patients in the presence of CM from unstimulated or cytokine-stimulated bone marrow human MSCs. Of note, both unstimulated and stimulated MSC-CM induced a significant reduction in tube formation of HSECs in comparison with control medium (aMEM). Further, we assessed the contribution of TIMP-1 in inhibiting angiogenesis of HSECs in the presence of human MSC-CM. To this purpose we performed tube formation assays of HSECs by adding anti-TIMP-1 blocking antibody to CM from both stimulated and unstimulated bone marrow hMSCs, using the appropriate isotype as control (Figure 7). Our results clearly show that TIMP-1 plays a fundamental role in the inhibition of angiogenesis also of liver sinusoidal endothelial cells.

Further, we investigated the contribution of TIMP-1 in controlling liver inflammation, by overexpressing TIMP-1 in MDR2 -/- mice by AAV9-mediated gene transfer and analyzed liver immune cell infiltration. AAV-TIMP-1 injection resulted in reduced liver infiltration of myeloid cells and did not affect Kupffer cells population. Moreover, TIMP-1 overexpression reduced the amount of neutrophils and infiltrating macrophages and increased the amount of restorative macrophages in comparison with control untreated MDR2 -/- mice. Further, AAV-TIMP-1 treated mice showed lower levels of bile acids, ALT and ALP than untreated MDR2 -/- mice (Figure 8), suggesting a beneficial effect of TIMP-1 overexpression in PSC mouse model. However, the AAV-LacZ injected mice used as AAV-infection control collectively showed less injury than untreated MDR2 -/- mice, suggesting that the observed effects were not specifically dependent on AAV9-TIMP-1. We also found that high levels of TIMP-1 negatively correlate with Treg accumulation in injured livers of MDR2-/- mice. Thus, we blocked TIMP-1 by administering an anti-TIMP-1 blocking antibody in MDR2 -/- mice. Nonetheless, TIMP-1 blockade did not result in amelioration of clinical score of liver injury, thus indicating that, although TIMP-1 plays an important role in dampening liver immunosuppression, other factors might contribute to liver injury in MDR2-/- mouse model (Figure 9).

Finally, we assessed the effects of extracellular vesicles (EVs) derived from human BM MSC in our PSC mouse model, finding that EVs from MSC might improve the clinical outcome of MDR2-/-mice.

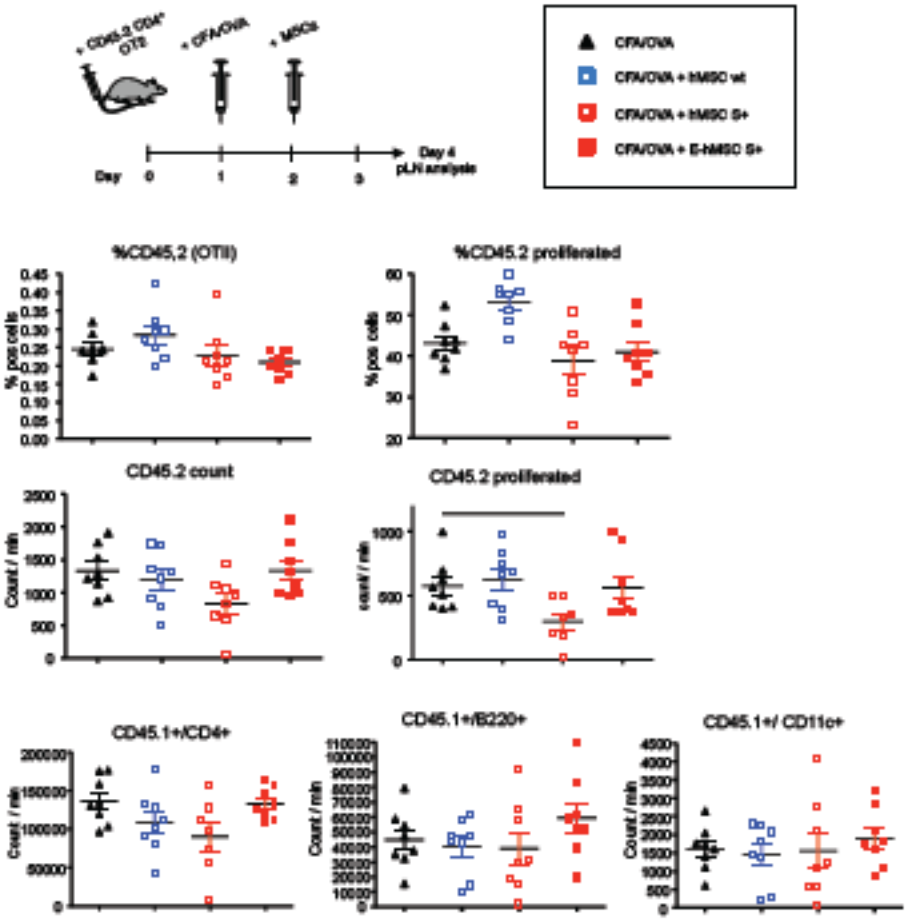


Figure 4 Effect of hMSC on OVA-specific T cell proliferation. The experimental protocol was designed to investigate the influence of hMSC upon the activation of OT62 (OVA6specific) T cells: OT2 CD45.2 CFSE6labelled cells (1x106) were transferred into CD45.1 C57 mice. After 24 hrs mice were immunized with OVA peptide (100 ng) in complete Freund s adjuvant (CFA) by s.c. injection into the footpad. On day 2 a group of animals received 500.000 hMSC wt injected s.c. in the back another group received 500.000 hMSC S+ s.c. and the last group received 500.000 encapsulated (E6)hMSC S+ s.c.. Immunized mice were sacrificed at day 4 the popliteal draining LNs (dLNs) were collected and cells were analysed by flow cytometry.

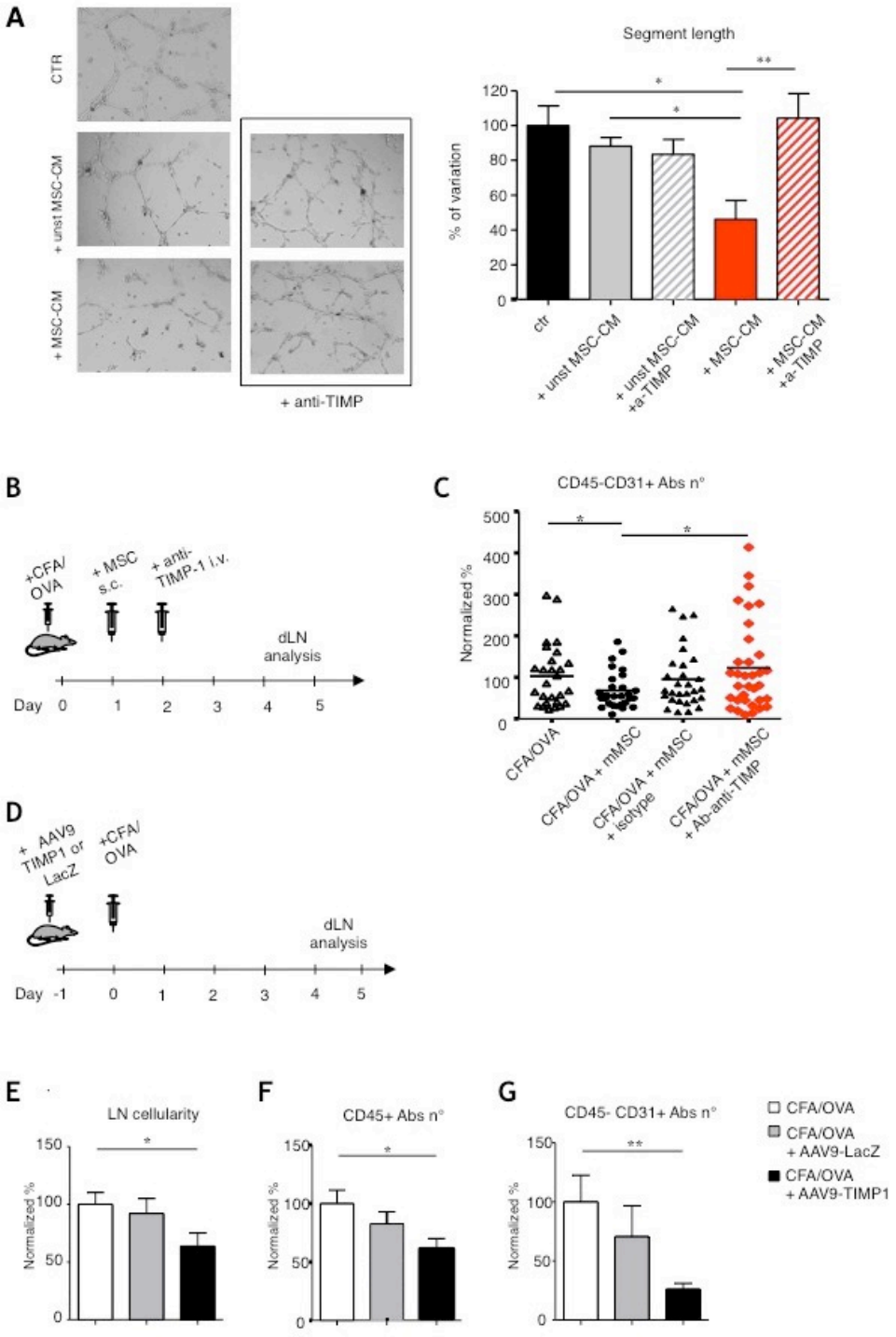


*Figure 5 TIMP-1 mediates the anti-angiogenic effect of MSC-CM in vitro and the anti6inflammatory effect of MSC in vivo.SVEC4-10 network formation in matrigel in the presence of MSC6CM or unst MSC6CM and anti mTIMP1 blocking antibody. A) Representative images at 6 hrs (left). Segment length quantification (right). Data are expressed as mean ± SEM (*p<0.05, **p < 0.01; One way Anova). B) Diagram of the experimental protocol designed to investigate the contribution of TIMP61 to the anti6inflammatory effects of MSC. Mice were immunized in the dorsal back with CFA/OVA on day 0 and, on day 1, 3 groups of animals received subcutaneous injection of 10^6 MSC in the lumbar back. 18 hours after MSC transplantation goat polyclonal anti-TIMP-1 IgG or isotype-matched goat IgG was i.v. administrated. On day 4 brachial LNs were collected, processed and analysed by flow cytometry; C) Graph showing the absolute number ofCD456CD31+ cells per single LN expressed as normalized percentage on CFA/OVA (3independent experiments, n=28 dLN). D) Diagram of the experimental protocol designed to over6express TIMP61 in immunized mice. One day after AAV9 administration (day 0),mice were immunized with CFA/OVA. Brachial draining LNs (dLNs) were collected 4days after immunization and digested for FACS analysis. E6G) Graph showing LNcellularity, absolute count of CD45+ and CD456CD31+ cells per single LN expressed as normalised percentage on CFA/OVA. In E6G error bars represent standard error, n= 6 mice for each condition (*p < 0.05; **p < 0.01; t-test*

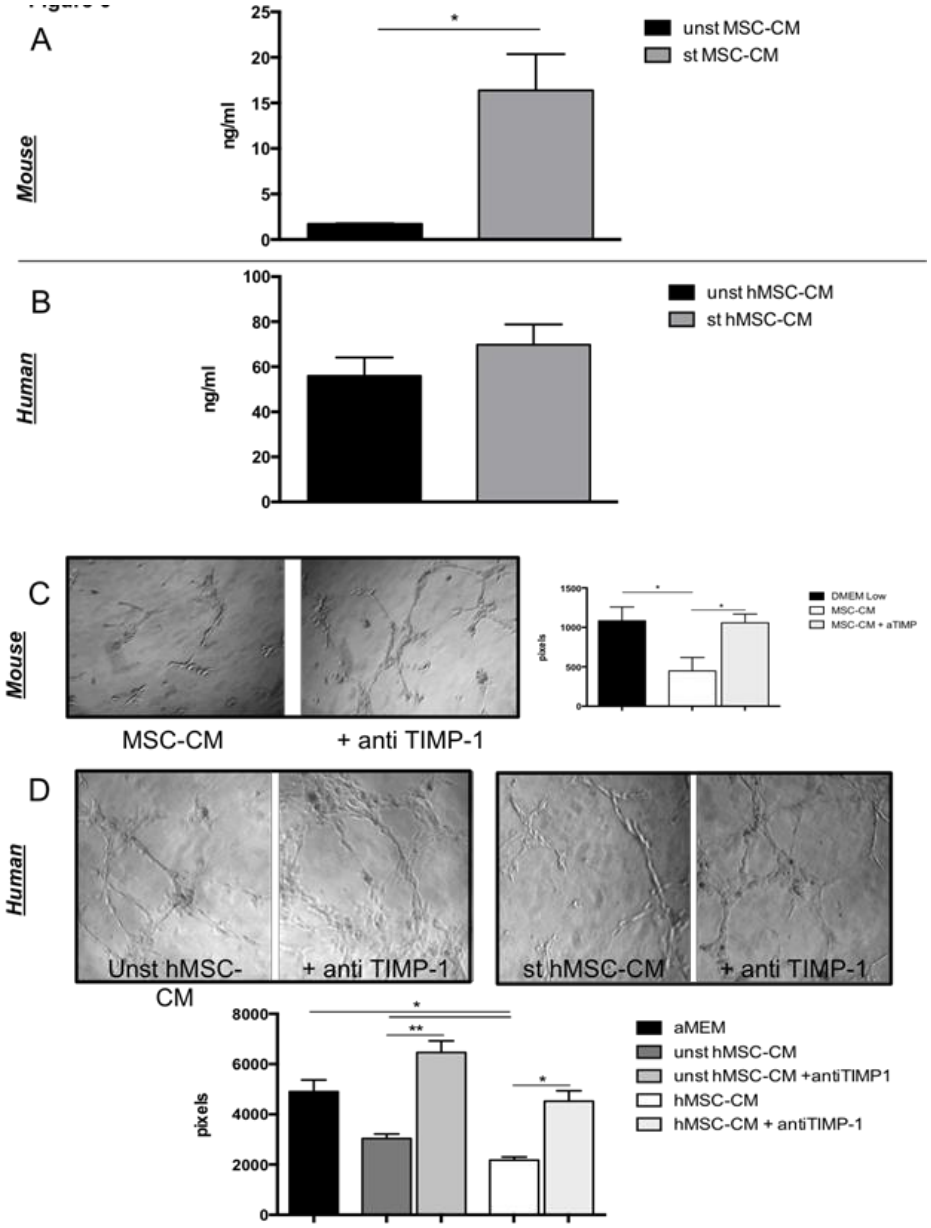
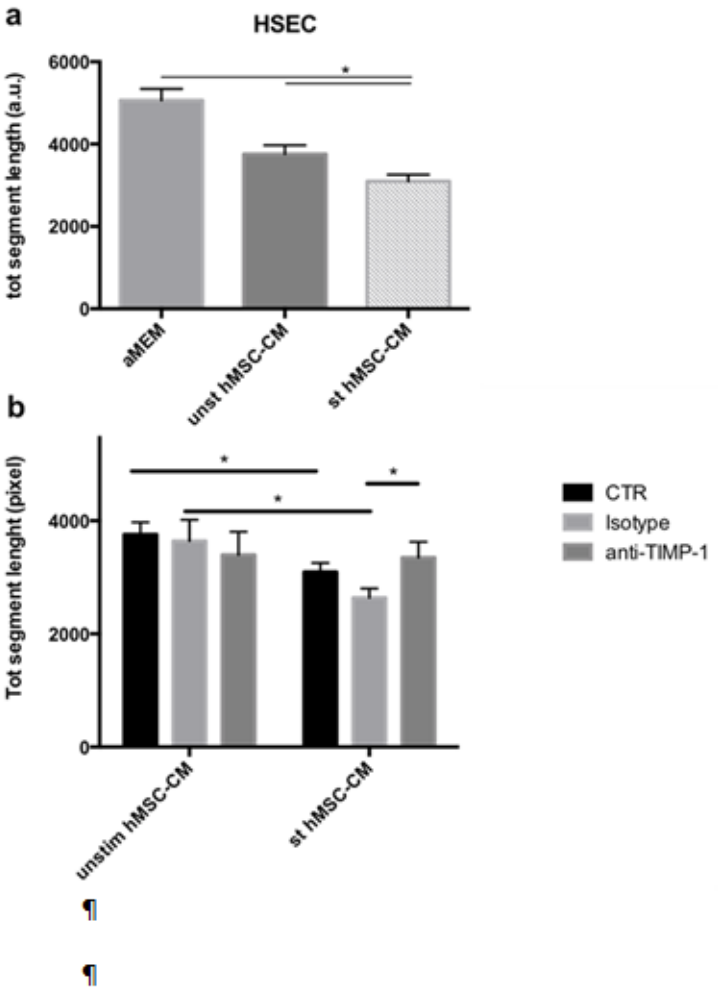


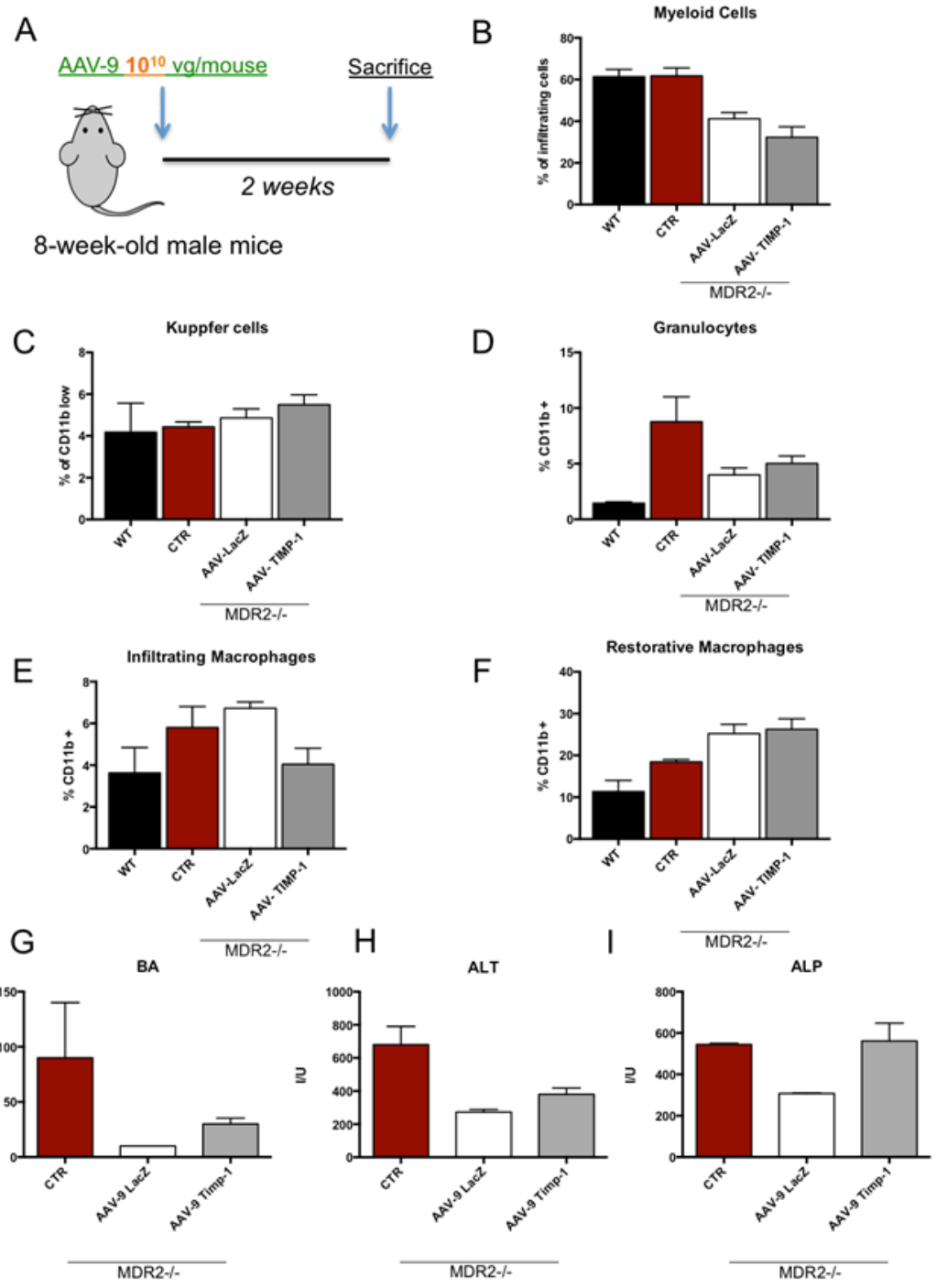
*Figure 6: Timp-1 blocking reverts the anti-angiogenic effect of mouse and human MSC conditioned media. MSC-derived TIMP-1 concentration in (A) mouse and (B) human unstimulated or stimulated MSC conditioned medium was measured with ELISA. Data are expressed as mean ± SEM (*p<0.05, parametric T Test). Tube formation assay was performed in the presence of (C) mouse or (D) human TIMP-1 blocking antibody. Representative images of (C) SVEC4-10 cell line or (B) Huvec cells are taken with a phase contrast inverted microscope at 4× objective magnifications. Graphs show the quantification of the tube segment length measured with ImageJ Angiogenesis Analyzer. Data are expressed as mean ± SEM (*p<0.05, **p<0.01; One way Anova).*

Figure 3



TIMP-1· plays· a· crucial· role· in· the· inhibition· of· angiogenesis· of· liver· sinusoidal· endothelial· cells.· MSC· were· plated· and· let· grow· until· confluence· in· ventilated· cap· flask.· Growth· medium· was· substituted· with· MEM· Alpha· with· Glutamax· supplemented· with· 2%· FBS,· 2mM· glutamine,· 100· U/ml· penicillin/streptomycin,· with· or· without· 25ng/ml· hIL1b,· 20ng/ml· hIL6,· 25· ng/ml· hTNFa· for· 24· hours.· Then· this· medium· was· changed· with· MEM· Alpha· with· Glutamax· supplemented· with· 2mM· glutamine,· 100· U/ml· penicillin/streptomycin· for· the· following· 18· hours.· Conditioned· medium· of· unstimulated· MSC· (unst· hMSC-CM)· or· stimulated· MSC· (st· hMSC-CM)· were· harvested.· (A)· The· angiogenic· effect· of· two· different· MSC· donors· was· tested· on· HSEC· from· one· PSC· patient· using· the· tube· formation· assay.· Matrigel· Matrix· (Corning)· was· thawed· O/N· at· 4°C· and· tips· and· 96-well· plate· flat· bottom· were· kept· O/N· at· 4°C.· The· day· of· the· assay,· 80µl· of· matrigel· were· seeded· in· the· 96-well· plate· and· left· to· polymerize· at· 37°C,· 5%· CO2· for· at· least· 30· minutes.· (B)· 1.3x104· HSEC· were· resuspended· in· 100µl· of· MSC· supernatant,· supplemented· with· 10%· FBS· with· or· without· isotype· control· or· anti-TIMP-1· 5ug/ml· (AF980· R&D),· seeded· on· the· solidified· matrix· and· incubated· for· 4hours· at· 37°C· 10%· CO2.· aMEM· with· 10%· FBS· was· used· as· positive· control.· At· the· end· of· the· incubation,· cell· tubes· were· imaged· at· 4×· objective· magnifications· and· analysis· was· performed· with· ImageJ· Angiogenesis· Analyzer.· Data· are· expressed· as· mean±s.e.m.· (*P<0.05,· **P<0.01;· one-way· ANOVA).¶

Figure 7



AAV-9- TIMP-1 infection does not result in specific amelioration of liver inflammation in MDR2-/- mice. The effect of TIMP-1 miming the MSC treatment was assessed by the over-expression of TIMP-1 mediated by the AAV-9 infection (AAV9-LacZ used as control; 10^{10} vg/mouse). Experimental schedule is represented in the panel **(A).** After two weeks from the infection, mice were sacrificed and liver and blood were harvested. Livers were mechanically and enzymatically (Collagenase II and DNAse I 0,2mg/ml at 37°C for 1hour) digested for FACS analysis of immune cell infiltration. Graphs showing myeloid Cells ($CD11b^+$ cells within the live cells) **(B),** Kupffer Cells (CD11b low, $F4\text{-}80^+$) **(C),** Granulocytes ($CD11b^+$, $LY6G^+$, $LY6C^+$)**(D),** Infiltrating Macrophages ($CD11b^+$, $F4\text{-}80^+$)**(E)** and Restorative Macrophages ($CD11b^+$, $F4/80^+$, $LY6C^-$ $LY6G^-$)**(F)** accumulation in MDR2-/- mice injected with PBS (CTR), AAV9-LacZ, AAV9-TIMP-1. Blood was processed to obtain serum and analyzed for the quantification of Bile Acid **(G),** ALT **(H),** and ALP **(I).** n= 4 mice per group, data are expressed as mean ± SEM (*p<0.05, **p<0.01, One way Anova).

Figure 8

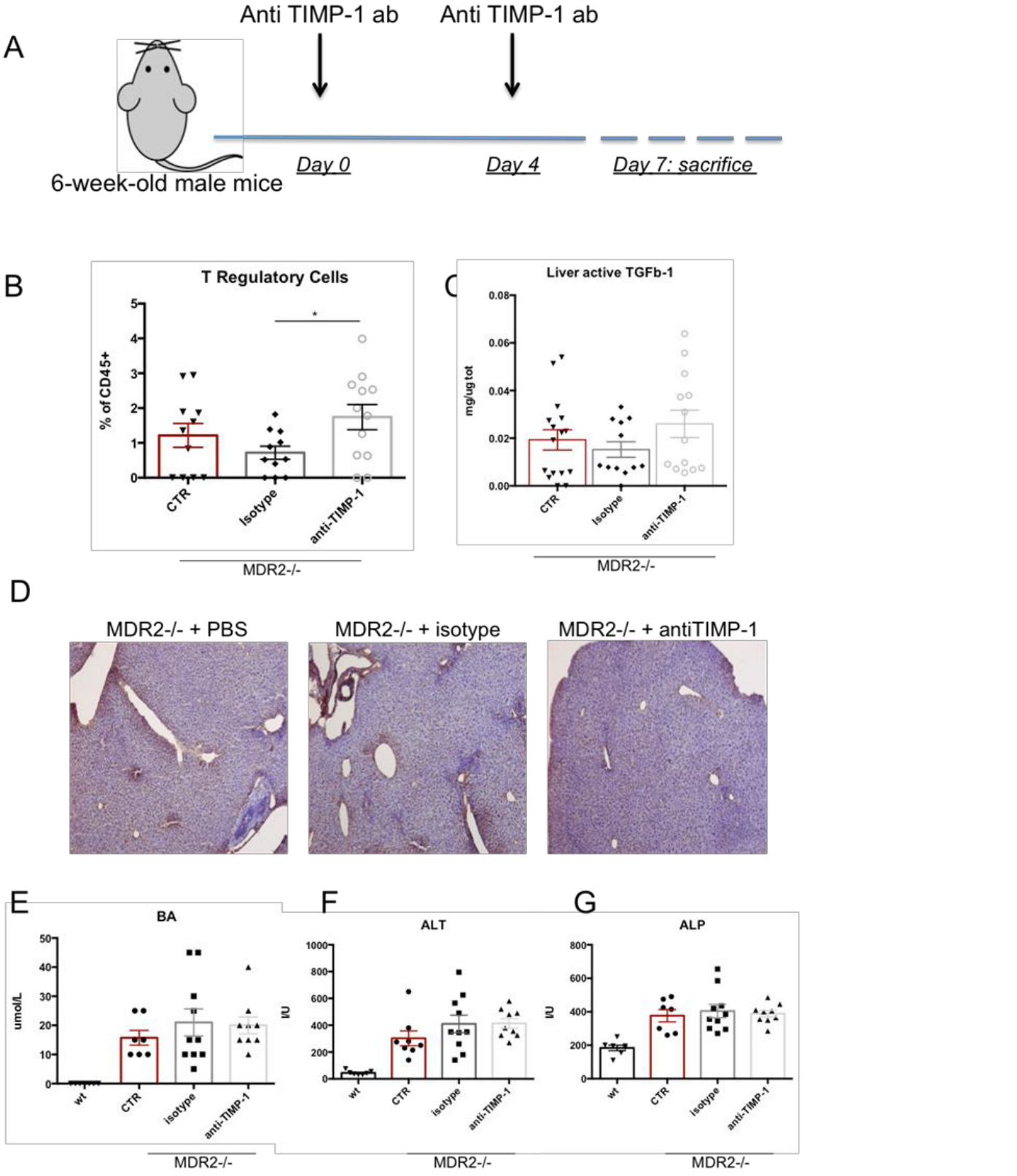


*Figure 9 TIMP-1 modulates regulatory T cells accumulation by regulating TGF beta activation in MDR2-/- mouse livers. The role of TIMP-1 in modulating liver inflammation was assessed by using anti-TIMP-1 blocking antibody. Experimental schedule is represented in the panel (A). 6-week-old MDR2-/- mice were i.p injected twice a week with anti-TIMP-1 blocking antibody, using isotype as control. Mice were sacrificed 7 days after the first injection and liver and blood were harvested. Livers were mechanically and enzymatically (Collagenase II and DNAse I 0,2mg/ml at 37°C for 1hour) digested for FACS analysis of immune cell infiltration. Graphs show regulatory T cells (CD45+ CD3+ CD4+ CD25+ FoxP3 + within the CD45+ leukocytes) (B) and amount of active TGF beta, measured by ELISA (C). In the panel D, IHC for CD31, marker of endothelial cells, show the altered liver tissue in MDR2-/- mice. Blood was processed to obtain serum and analysed for the quantification of Bile Acid (E), ALT (F), and ALP (G). n= 4 mice per group, data are expressed as mean ± SEM (*p<0.05, **p<0.01, One way Anova).*

1.3.4.3 WP4 Conclusion

Exploiting a mouse model of local inflammation, we demonstrated that MSC inhibit high endothelial cell activation and lymphocyte homing to lymph nodes. In order to clarify MSC mechanism of action, we performed a high-throughput analysis of MSC secretomes that provided us a plethora of pharmacologically targetable molecules for the treatment of inflammation. Among the upregulated proteins, we focused our attention of TIMP-1, providing evidence that TIMP-1 secreted by MSC plays a fundamental role in the inhibition of angiogenesis in vitro and in vivo, inhibiting T-cell homing into inflamed lymph nodes.

Importantly, we found a common antiangiogenic signature in the secretome from both mouse and human MSC stimulated with proinflammatory cytokines. In agreement with data obtained with mouse MSCs, we also showed that soluble factors secreted by human MSCs affect the ability of HUVEC cells to form tubes. Interestingly, in the case of human cells, MSC-CM was able to inhibit tube formation even when MSC had not been primed by cytokines. However, pre-activation with pro-inflammatory cytokines strengthened the anti-angiogenic effects of MSC-CM, thus supporting our hypothesis that, during an inflammatory response, licensed MSC target angiogenesis and thus dampen the inflammatory response.

Collectively, our results clearly show that TIMP-1 plays a fundamental role in the MSC-mediated inhibition of angiogenesis in vitro and in vivo model of local inflammation. We confirmed our findings by exploiting mouse and human MSC, as well as both mouse ad human endothelial cells. Importantly, we found that TIMP-1 plays a fundamental role in the inhibition of angiogenesis also of liver sinusoidal endothelial cells from PSC patients. However, TIMP-1 AAV9 mediated gene transfer in MDR2-/- mice resulted in only a partial beneficial effect. Of note, this effect seemed also to be independent from TIMP-1 and likely associated with AAV vectors injections.

Nevertheless, although high levels of TIMP-1 negatively correlate with Treg accumulation in injured livers of MDR2-/- mice, TIMP-1 blockade did not result in amelioration of clinical score of liver injury in MDR2-/- mice, thus indicating that other factors might contribute to liver injury in this mouse model. Finally, we found that extracellular vesicles (EVs) derived from human BM MSC might improve the clinical outcome of MDR2-/-mice.

1.3.5 WP5 Genetic, biological & pharmacological enhancement of human ORB1+MSC re liver inflammation treatment.

1.3.5.1 WP5 Introduction

The overall objectives of WP5 were:

- To isolate, expand, and cryopreserve CD362+MSC and PA-MSC from human bone marrow and umbilical cord tissues under GLP conditions.
- To provide GLP-compliant MERLIN SOPs and BMRs to partners to define, govern and record the thawing and expansion of cryopreserved human ORB1+MSC and PA-MSC in each WP laboratory.
- To assess the impact of thawing/washing of cryopreserved stromal cells upon efficacy in CCl4-induced model of liver Injury.
- To assess the impact of prolonged cell culture passage upon MSC efficacy in vitro and in CCl4-induced model of liver Injury.
- To determine the impact of ORB1-overexpression upon MSC efficacy in CCl4-induced model of liver injury.
- To explore unmodified and 'optimised' ORB1+ MSC in CCl4-induced model of liver injury.

1.3.5.2 WP5 Key Work Undertaken and Results Achieved

In Period 1, WP5 focussed on the manufacture of cryopreserved CD362+ MSC and PA-MSC from human bone marrow and umbilical cord tissue - and delivered cells to partners in WPs 1, 3, 4, 5 and 6 for testing. >200 vials of cryopreserved CD362+, and PA-MSC from human marrow and umbilical cord were delivered to MERLIN partners for pre-clinical experiments.

In addition, studies were completed that show no impact of cryopreservation/thawing on CD362+ MSC efficacy in the CCl4-induced model of liver injury in the C57Bl6 mouse.

The impact of the glucocorticoid Budesonide on the efficacy of ORB1+ MSC in suppressing T lymphocyte proliferation was also assessed. Pre-treatment of CD362+ MSC with Budesonide stimulates expression of the CD362 protein, GILZ mRNA and TIMP1 RNA. Notably, Budesonide-treated ORB1+ MSC significantly improved MSC-mediated suppression of T lymphocyte proliferation.

In total ORB delivered over 400 vials of cryopreserved stromal cells to MERLIN partners over the course of Period 1 and Period 2. ORB explored novel activators of ORBCEL-C and focused on developing Budesonide as an activator of ORBCEL-C.

Researchers have worked on enhancing the manufacturing of the ORBCEL-C product using the MACSQuant Tyto to select near 100% pure stromal cells and expanding ORBCEL-C on the closed automated Quantum Bioreactor and this led to a collaboration between ORB and NHBST to test Quantum expanded ORBCEL-C in the REALIST clinical trial (https://clinicaltrials.gov/ct2/show/NCT03042143)

ORB have explored the potency of ORBCEL-C with the CD4 T cell proliferation assay and VCAM1-specific Endothelial cell activation assay and discovered a biomarker (ORB2) that allows ORB to select effective cord tissue donors for ORBCEL-C Manufacture.

ORB collaborated extensively with NHSBT and UOB over the entire MERLIN project to transfer the ORBCEL-C Manufacturing process to NHSBT for the MERLIN Phase 2a clinical trial. This has allowed the MERLIN trial to progress to treat its PSC patient in March 2019.

https://www.prnewswire.co.uk/news-releases/clinical-trial-investigates-orbsen-therapeutics-orbcel-c-tm-immunotherapy-as-treatment-for-two-autoimmune-liver-diseases-820613487.html

Finally, ORB have shown that ORBCEL-C derived extracellular vesicles express CD362, CD39 and CD73 and retain intrinsic ATPase activity. With a view to understanding the consistency of the ORBCEL-C product, ORBCEL-C derived from 7 donors in 8 series of experiments was tested in a rat model of lung inflammation and showed consistent function of the ORBCEL-C Drug Product.

1.3.5.3 WP5 Conclusion

MERLIN WP5 enabled ORB to develop the ORBCEL-C selection and GMP manufacturing process from the pre-existing ORBCEL-M marrow CD362-selection & expansion process developed in the REDDSTAR program. ORB then manufactured and distributed >400 vials of ORBCEL-C for pre-clinical testing, optimisation and in vitro evaluation in WP1, WP3, WP4, WP5 and WP6. ORB have tech transferred the ORBCEL-C Manufacturing process to NHSBT in WP7 to allow clinical manufacture of ORBCEL-C for the MERLIN clinical trial in WP8. ORB have established the 2 year shelf life of the ORBCEL-C Drug Product. ORB have since established GMP manufacturing operations and are collaborating with UOB to design a Phase 2b MERLIN2 Adaptive Licensing in patients with autoimmune liver disease.

1.3.6 WP6 Advanced tools for bio-distribution of MSC.

1.3.6.1 WP6 Introduction

BioInVision, based in Cleveland, Ohio, USA, offers imaging instrumentation and methodologies critical to preclinical studies. The unique CryoViz™ instrument, utilizing the patented cryo-imaging technology, allows microscopical anatomical and molecular fluorescence imaging of laboratory small animals such as a mouse or organs excised from them with single-cell sensitivity. With its sub-ten-micron-scale imaging, cryo-imaging allows one to detect even single stem or cancer cells anywhere in a mouse. The technology is also offered as a service and is targeted to a variety of biomedical applications including stem cell homing and biodistribution, cancer metastasis, imaging agents, drug discovery and delivery, tissue engineering, mouse phenotyping etc. BioInVision's cryo-imaging technology was a good match for the MERLIN project which focused on pre-clinical and clinical studies to investigate specifically the use of MSCs as a therapy for Primary Sclerosing Cholangitis (PSC), a type of liver disease for which there is currently no cure. In MERLIN, BioInVision partnered with several EU institutions developing stem cell-based therapies specifically targeting the inflammatory components of liver disease. BioInVision's cryo-imaging technology powered by the CryoViz™ instrumentation and software was employed to provide high-resolution, microscopic colour anatomical and molecular fluorescence volumetric imaging, accurate stem cell detection, manual review and editing of cell detection, probabilistic segmentation of organs in 3D from anatomical and fluorescence volumes, global and local cell quantification, and 3D visualization of stem cell biodistribution within mouse-sized volumes. Together, MERLIN consortium partners and BioInVision set out to develop MSC therapy for liver disease.

1.3.6.2 WP6 Key Work Undertaken and Results Achieved

For MERLIN, BioInVision customized the CryoViz™ instrument to support (i) high throughput cryo-imaging of multiple-sample-arrays within the same frozen block, (ii) smart image acquisition that employs a tissue detection algorithm for skipping over unnecessary regions during imaging thereby reducing imaging time, (iii) multi-fluorophore stem cell detection that enabled tracking of multiple populations of stem cells as well as immune cells within the same specimen, and (iv) semi-automatic organ/tissue segmentation that aided in analysing local biodistribution of cells within specific organs and tissues. In software, BioInVision developed the MERLIN Biodistribution Explorer (MBE), a tool for analysing large volumes of cryo-imaging data, thereby complementing the hardware modifications to the CryoViz™. MBE supports profile-based access privileges upon login, structured browsing of experimental data organized by work package, institution, lead investigator, and imaging study, browsing of metadata, 3D graphics and movies, and text comments relevant to an imaging study, and live data viewing in 2D/3D in an interactive fashion. MBE is augmented by an excellent set of software modules that provide high-throughput stem cell detection, manual review and editing of detection results, global and local quantification of cells, probabilistic 3D segmentation of organs and tissue, and 3D visualization of stem cell biodistribution within mouse-sized volumes. Together, the modified CryoViz™ system and software were employed in several work-packages of MERLIN, which we describe in the next paragraphs.

First, in a study with UNIPD, CryoViz™ was used to investigate the mechanism of action of MSC (WP4). Mesenchymal stem cells (MSC) represent a promising therapeutic approach in many diseases in view of their potent immunomodulatory properties, which are only partially understood. Although MSC seem to be effective in treating diverse immune-mediated disorders, a unifying mechanism of action was missing. In a previous study, the proteomic analysis of the MSC secretome identified the tissue inhibitor of metalloproteinase-1 (TIMP-1) as a potential effector molecule responsible for the anti-angiogenic properties of MSC. Using CryoViz™ cryo-imaging and cell dispersion analysis module within MBE, it was verified that subcutaneously injected MSC did not migrate away from the site of injection during the experimental time (5 days), both in immunized and in untreated mice (Figure 10). Together with the previous study, these data indicate that MSC are able to dampen inflammation through the release of soluble mediators. This study of the broad anti-inflammatory properties of MSC paves the way for developing strategies that exploit MSC-mediated inhibition of lymph node angiogenesis in the treatment of inflammation-associated pathologies.

Second, in collaboration with EMC, the immunogenicity of MSC was examined (WP3). Mesenchymal stromal cells (MSC) possess immunomodulatory properties and are low immunogenic, both crucial properties for their development into an effective cellular immunotherapy. They have shown benefit in clinical trials targeting liver

diseases, however the efficacy of MSC therapy would benefit from improvement of the immunomodulatory and immunogenic properties of MSC. In this cryo-imaging study, the responsiveness of ucMSC to *in vitro* optimisation treatment was studied. By employing 3D visualization and quantification analysis of biodistribution from whole mouse cryo-imaging volumes, we observed that four hours after intravenous infusion in mice with CCl4-induced inflammatory liver injury, the majority of ucMSC were still trapped in the lungs. Rapid clearance of ucMSC(VitB6), ucMSC(Starv+VitB6) and ucMSC(MC) and altered bio-distribution of ucMSC(TGFβ) compared to untreated ucMSC was also observed (Figure 11). This study confirmed that majority of infused ucMSC accumulate in the lungs and that there is a rapid clearance of cells, with the cells mostly moving to the liver, an important finding that would inform future cellular immunotherapy strategies.

Third, in partnership with UoB, the efficacy and biodistribution of MSC in both acute and chronic mouse models of PSC was studied (WP1). The ability of mesenchymal stromal cells (MSC) to immunomodulate offers therapeutic potential but the inherent heterogeneity of unsorted MSC populations may explain varied/reduced function and poses regulatory challenges. Thus, in this study, the efficacy of purified CD362+ MSC in relevant pre-clinical models of PSC as a prelude to a clinical trial was examined. A time-course of bio-distribution was determined by whole mouse cryo-imaging of Q-dot labelled MSC, followed by MBE analysis. A seven-day time-course indicated MSC were cleared rapidly although there was a liver-specific increase in MSC 2-3 days after infusion (Figure 12). We concluded that CD362+ human MSC exert a potent anti-inflammatory action in murine models of PSC.

Last, in partnership with ORB, homing and biodistribution of ORB1+ MSC was studied upon injection into healthy and CCl4-induced liver injury mice using CryoViz™ cryo-imaging (WP5). First, ORB1+ MSC were isolated, expanded, and cryopreserved. Subsequently, they were injected into the two groups of mice - healthy and injured - and their homing and biodistribution was studied using MBE software. In the first experiment, mouse MSC from UBC-eGFP mice (green) were injected into FAP-DTR BAC Tg mice (low and high doses) to study homing and distribution. The aim of the experiment was to see if ablating FAP+ cells with DTX in the FAP-DTR mouse altered the engraftment distribution of the GFP+ MSC. In a second experiment, PKH26 (red)-labelled MSC were injected in uninjured and acute CCL4 injury mice to study distribution, homing and survival. It was found that cell retention and homing to affected organs was higher in the injured group as compared to the control group. (Figure 13).

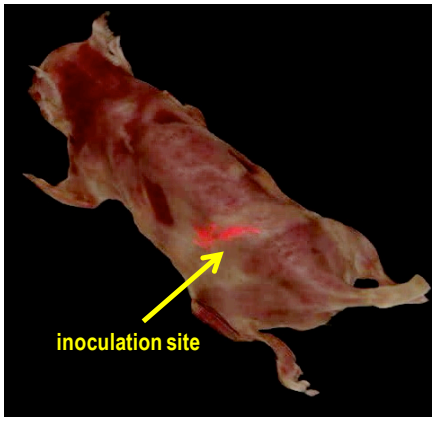
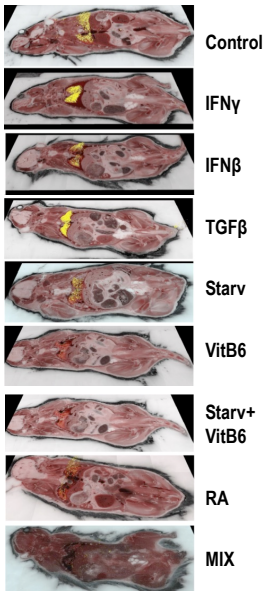


Figure 10

Mechanism of Action. Using whole-mouse cryo-imaging and cell dispersion analysis within MBE, we verified that subcutaneously injected MSC did not migrate away from the site of injection during the experimental time (5 days), both in immunized and in untreated mice. Together with a previous study, these data indicate that MSC are able to dampen inflammation through the release of soluble mediators.



Immunogenicity of MSC. Tracking of labeled ucMSC by CryoViz enables in depth visualization of MSC after administration and subsequently gives an interpretation of not only their bio-distribution, but also their survival. In a previous study, localization in the lungs and short survival time of intravenous injected bone marrow derived MSC was observed. In the present study, it was observed that four hours after intravenous infusion in mice with CCl4-induced inflammatory liver injury, the majority of ucMSC were still trapped in the lungs. Rapid clearance of ucMSC(VitB6), ucMSC(Starv+VitB6) and ucMSC(MC) and altered bio-distribution of ucMSC(TGFβ) compared to untreated ucMSC was also observed. This study confirms that the majority of infused ucMSC accumulate in the lungs and that there is a rapid clearance of cells, an important finding that would inform future cellular immunotherapy strategies.

Figure 11

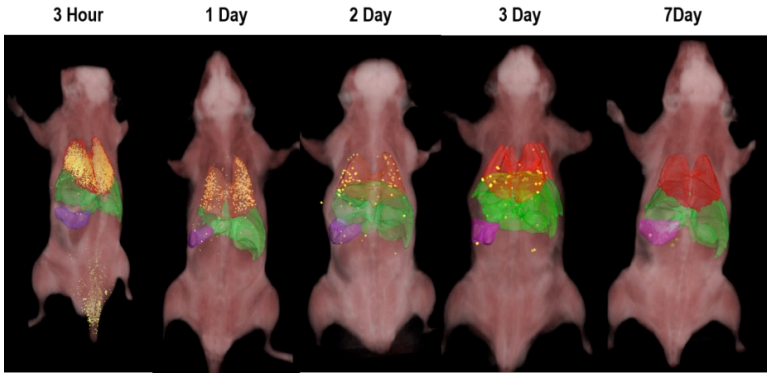


Figure 12

Efficacy and biodistribution of MSC in mouse models of PSC. A time-course of biodistribution was determined by whole mouse cryo-imaging of Q-dot labelled MSC, followed by MBE cell detection and quantification. A seven-day time-course indicated that MSC were cleared rapidly although there was a liver-specific increase in MSC 2-3 days after infusion. MSC are cleared rapidly after infusion despite modest hepatic homing and significant M2 polarisation of hepatic monocytes. We concluded that CD362+ human MSC exert a potent anti-inflammatory action in murine models of PSC.

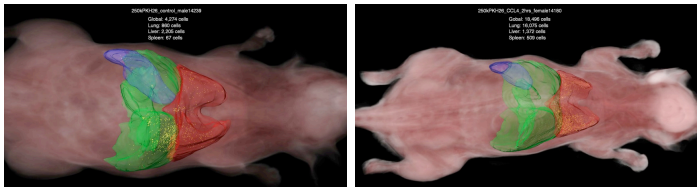


Figure 13

Biodistribution of ORB1+ MSC in healthy versus CCL4-induced liver injury mice. Homing and biodistribution of ORB1+ MSC was studied upon injection into healthy and CCl4-induced liver injury mice using CryoViz™ cryo-imaging (WP5). First, ORB1+ MSC were isolated, expanded, and cryopreserved. Subsequently, they were injected into the two groups of mice and their homing and biodistribution was studied using MBE software.We found that cell retention was higher in experimental (injured) mice as compared to control (uninjured) mice.

1.3.6.3 WP6 Conclusion

The MERLIN project aimed to develop a therapy using purified MSC populations that alleviates the ongoing inflammation that drives fibrosis in liver. Prior to MERLIN, there was lack of evidence and concerns regarding human PA-MSC survival and persistence upon transplantation into mice. The research faced unknowns such as retention period of MSC anti-inflammatory behaviour, whether there was differentiation upon infusion, the molecular mechanisms underpinning efficacy, immuno-modulatory action *in vivo,* etc. Pre-clinical imaging experiments on the CryoViz™ conducted by BioInVision along with MERLIN partners through several work-packages (WP1- UoB, WP3-EMC, WP4- UNIPD, WP5-ORB) have helped provide some answers, especially pertaining to MSC homing and biodistribution upon transplantation into injured and healthy mice. This knowledge is enabling the MERLIN consortium partners to better understand the mechanism of action of the MSCs in mouse models. The study results will help optimize the MSC type to be administered, develop cell preparation methods, and optimize the dosage strategy. All the pre-clinical findings will enable a successful Phase 1/2 clinical safety trial in patients with PSC.

1.3.7 WP7 Generation of Investigational Medicinal Product Dossier and Manufacture of Clinical Grade ORB1+/- MS

1.3.7.1 WP7 Introduction

The aim for NHSBT has been to develop a GMP compliant process for the manufacture of clinical grade CD362+ve enriched Mesenchymal Stromal Cells from umbilical cord tissue and provide these cells in approved batches for a clinical trial. This work has included completion of development and engineering runs plus validation of standardized QC testing and final release criteria in place. NHSBT has also undertaken a stability study on the Drug Product. The NHSBT manufacturing facility in Birmingham, UK, has an MHRA license for this work and the results informed the Clinical Trial Application which included the development of an IMPD and Investigator Brochure. The Drug Product is produced directly from the Drug substance and cryopreserved in aliquots of 40 x10^6 or 80 x10^6 cells per bag, ready for issue and transport to the ward and then thawing ahead of administration to patients.

1.3.7.2 WP7 Key Work Undertaken and Results Achieved

From the start of the grant NHSBT worked closely with the other partners on the pre-clinical development and small-scale cell culture work as part of a technology transfer process to enable future production of MSCs under GMP by NHSBT. The NHSBT manufacturing unit along with the NHSBT Quality Assurance Dept reviewed the process design, equipment, proposed consumables and reagents to ensure that a full-scale process could be developed in compliance with MHRA regulations. Optimisation of QC assays, preparation of SOP and BMR documentation and equipment Installation and Operational Qualification validation studies were carried out.

Extensive work was undertaken to develop a consistent supply of Bone Marrow as a source of MSCs. As this proved challenging, the decision was taken to switch the source material for MSCs to *umbilical cord* (UC) tissue of which NHSBT has ready access to via the NHS Cord Blood Bank.

The development of a cell selection protocol to enrich for CD362+ cells from UC was undertaken in the first period although this subsequently required additional work to ensure the process met GMP standards. A significant achievement in period 2 was the development of a complete process for MSC production from UC tissue that could achieve MHRA approval. The development and validation of this process formed the bulk of the contribution from NHSBT towards the preparation of the initial IMPD and the Clinical Trial Application.

The manufacturing process required extensive development for the cell selection and cell expansion steps. This resulted in some delays and in particular, the process of selecting viable CD362+ cells from umbilical cord tissue proved difficult to develop under GMP conditions, with many of the materials not being available as CE marked for clinical use. In addition, a problem was later identified with a cell dissociation device used to assist the enzyme digestion of the UC tissue. Therefore, further development and validation work was undertaken during P3 to ensure that a new rocker device could be used in a temperature controlled incubator as a suitable alternative for tissue digestion ahead of the subsequent CD362+ cell enrichment. Thus, while important challenges were encountered, they were ultimately overcome.

Methodologies for testing of the Drug Substance and Drug Product ahead of QP release were finalised during P3. The testing included Endotoxin testing, cell counts and morphology for the Drug Substance and flow cytometry for MSC phenotype, sterility, mycoplasma testing and karyotyping for the Drug Product.

As the overall process to produce cells for the clinical trial was delayed a no-cost extension was agreed to run for a year from January 2018.

Following the problems with tissue dissociation a number of additional full-scale validation runs were undertaken using the new method in the GMP compliant facilities of NHSBT Birmingham. A stability programme was also developed for the Drug Product and for the transport of the Drug Product to the clinical units.

The problems identified with the tissue dissociation device and the need to develop another process for this stage of manufacturing meant there was a need to submit a revised IMPD to the MHRA. Once the approval for the amendment was received from the MHRA the manufacture of clinical grade MSCs for use in the clinical trial was finalised and

NHSBT then started to prepare cryopreserved Drug Product batches with in-house QP release for clinical use from June 2018. The process takes many weeks to expand sufficient cells from the low numbers of CD362+ enriched cells derived from umbilical cord tissue. Each batch is produced from final cell cultures in multiple Cell Stack flasks after Passages through a number of smaller cell culture plates and flasks. The Clinical trial opened in late 2018 for patients with PSC and AIH and will therefore run beyond the end of the P4 extension period. NHSBT has produced many of the batches needed for the trial during P4 and is committed to producing the final batches required in 2019.

1.3.7.3 WP7 Conclusion

The Clinical trial opened in late 2018 for patients with PSC and AIH and so the primary endpoints of the grant have been met. A number of difficulties were encountered during the grant and lessons learned by NHSBT are primarily focussed on the difficulties in translating an R&D process into GMP compliant methodology. Many of the R&D starting procedures did not lend themselves easily to a standard GMP process that can be undertaken by a range of staff within a GMP facility. In addition, many of the essential reagents did not have CE marking and confirming these could be used under GMP was a time-consuming process. Linked to this, the later failure of a key piece of equipment to function effectively under GMP delayed the project extensively and resulted in the need to repeat many of the process development steps and validations. The final process developed is however now delivering the Drug Substance and Drug Product batches `required although it is not straightforward and will not lend itself easily to any future scale up.

The MERLIN project has been instrumental in creating an experienced team of staff within NHSBT capable of undertaking similar early phase tech transfer and development projects more readily in the future. The team has become more experienced and knowledgeable both in terms of GMP compliant manufacturing and also in the requirements of the Clinical team to gain success in an early phase clinical trial of a novel cell therapy.

1.3.8 WP8 Phase 2a clinical trial of ORB1+/- MSC in patients with PSC

1.3.8.1 WP8 Introduction

The ultimate goal of the MERLIN project is an open label clinical trial delivering CD362 selected umbilical cord derived, mesenchymal stromal cells, to patients with immune mediated liver disease. The clinical trial team remains ready to start this process, which is always challenging. Fundamentally this WP has remained live and with all the components ready to start the trial but technical challenges have delayed our progress. These technical challenges are inevitable when one proposes a novel experimental medicine study of a cell therapy, such as is planned. Participant safety is paramount and therefore the trial must be on hold until the manufacture of clinical-grade product is complete and all related regulatory approvals are in place. This appears to be close which is inevitably exciting for all.

1.3.8.2 WP8 Key Work Undertaken and Results Achieved

We generated the relevant regulatory paperwork for the Merlin trial including the study protocol, culminating in formal MHRA and Ethics submissions. D8.1 (Clinical Trials Authorisation document for the Medicines and Healthcare Products Regulatory Agency (MHRA)) was submitted in Period 2. This included an updated approval from the MHRA, in view of changes to the protocol.

The clinical trial is ongoing; the first patient was treated on 29 March 2019.

University of Birmingham trials a new stromal cell immunotherapy for chronic liver disease

Posted on 29 Mar 2019

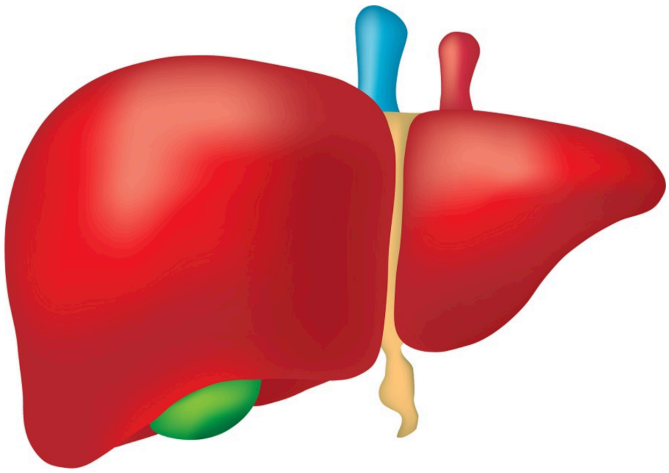
Share this page

The University of Birmingham has launched a trial which could lead to a ground-breaking new way of treating people with two types of chronic liver disease.

Up to 56 patients are being recruited to take part in the MERLIN trial, which will investigate the safety and efficacy of a new cellular immunotherapy in patients with either Primary Sclerosing Cholangitis (PSC) or Autoimmune Hepatitis (AIH).

Both PSC and AIH involve inflammation of the bile ducts, which can result in significant liver damage and many of those affected end up needing a liver transplant. Current options for treating PSC and AIH are limited.

The new cell therapy being trialled is a single infusion of specially selected mesenchymal stromal cells (MSCs) which will be administered to participants. The first patient has recently received the cells at Queen Elizabeth Hospital, Birmingham, which is run by University Hospitals Birmingham NHS Foundation Trust.



Up to 56 patients are being recruited to take part in the MERLIN trial, which will investigate the safety and efficacy of a new cellular immunotherapy in patients with either Primary Sclerosing Cholangitis or Autoimmune Hepatitis

Figure 14 Press Release for the Start of Clinical Trial (UoB)

1.3.8.3 WP8 Conclusion

WE were able to overcome the many challenges of obtaining regulatory approval for the trial and successfully manufacturing MSC and opened the clinical trial in Dec 2018. We have recruited multiple patients and treatment thus far has gone well with no adverse effects. We will support the delivery of the trial with other resources and look forward to completing the trial by late 2020.

1.3.9 WP9 Data and Samples Management

1.3.9.1 WP9 Introduction

The multi-disciplinary nature of MERLIN meant that many different types of data were created and analysed throughout the project. To ensure that data were securely but openly shared, and that the movement of samples was efficiently tracked between partners, we established suitable online infrastructure, based on PT's StudyVault platform technology.

1.3.9.2 WP9 Key Work Undertaken and Results Achieved

Data Management

During Period 1, working with the MERLIN data partners, a bespoke customisation of StudyVault was established for use in the project - StudyVault-MR. PT technologists worked with the data partners on the co-design (task 9.1), configuration (task 9.2), and installation and validation (task 9.3) of the system. On-going software support was also provided (task 9.4). The specific and unique requirements of MERLIN meant that very substantial customisation of the system was required, with some adjustment of the underlying architecture and data handling models.

StudyVault was established online and accessed via https://www.studyvault.eu/. All research data was held in an encrypted format on the cloud (there was no personal patient data created or shared on the system). Data security and confidentiality were key features. While all MERLIN data was pre-clinical research data, and so no personal or human-related data was stored, StudyVault is engineered to support compliant, secure storage of clinical data.

Co-design, installation and validation of the bespoke system was completed in the first years of the project. On-going support and any adjustments were delivered using a continuous delivery approach (so adjustments and customisations of the data platform did not hinder the performance of the live system). Significantly we added support for large-volume data formats from molecular biology platforms.

Key deliverables D9.1 Data Platform Configuration Report and D9.2 Data Platform Verification Report were completed and submitted in period 1.

During periods 2 to 4, the WP9 team continued to provide support to data partners, uploading additional data as required and making improvements to the StudyVault-MR system.

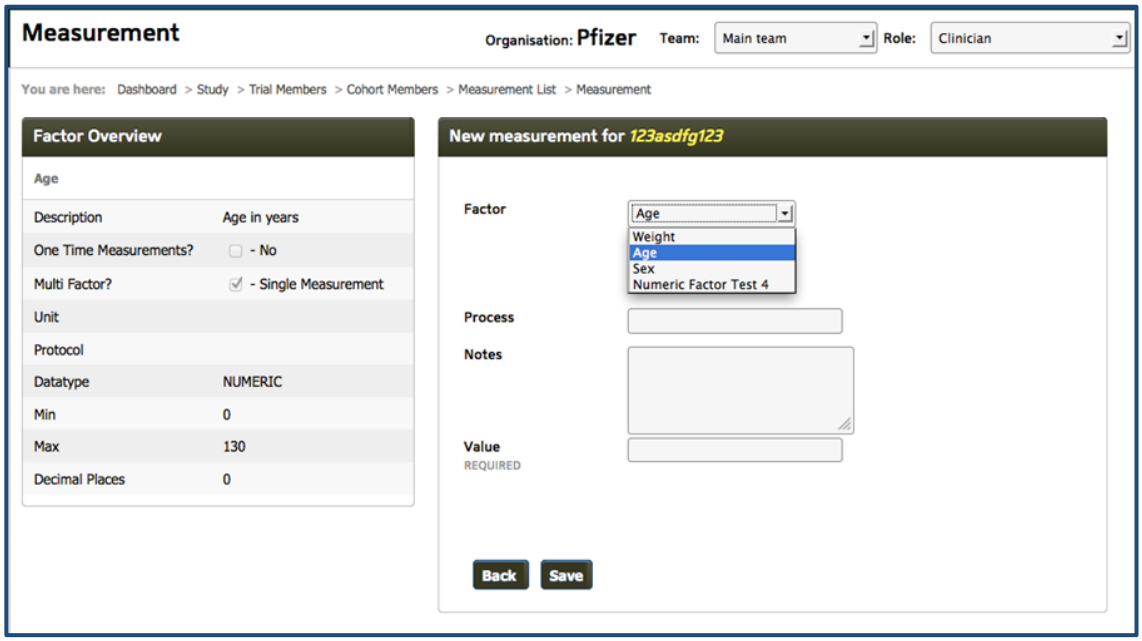


Figure 15 Example StudyVault screen

Samples Management

The management and monitoring of the movement of samples from one partner facility to another is a critical process, which has been found to frequently cause issues in life sciences projects. A key aim of WP9 was to marshal the samples management process and ensure smooth delivery of samples requirements.

Following liaison between the partners most involved in the creation and shipping of samples, an infrastructure for samples management in MERLIN was established in period 1.

During period 2, samples were transferred and managed for pre-clinical studies and sample transfers proceeded smoothly. There was a review of samples transfer logistics and some changes were introduced. The MERLIN Master Samples Register was produced. Partners opted for direct contact as the central approach for samples management, with an additional layer of oversight provided by collecting samples transfer data (with scope for partners to highlight any problems). A samples subgroup was established within the consortium to deal with any issues arising.

Samples during period 3 were dealt with and managed for pre-clinical studies without any issues arising. Direct contact between partners remained the central approach. The Master Samples Register for period 3 captured details of samples transfers, as well as protocols for sample preparation prior to transfer, storage on arrival and handling prior to use.

The Master Samples Register was maintained throughout RP4. Throughout the project, the management of samples and their transport went smoothly, and common issues of samples tracking were avoided.

1.3.9.3 WP9 Conclusion

The establishment and maintenance of the StudyVault system addressed the key challenge of multi-format, multi-owner data management in MERLIN. The system provided the facilities and supports required by the life-sciences partners, while maintaining data security and integrity.

The samples management system also worked as planned, and ensured that MERLIN avoided common research project issues and delays around ownership, location and status of samples.

1.3.10 Main S&T Results: Conclusion

As will be evident from the above, MERLIN has generated new knowledge about the efficacy, mechanisms of action, immune response, optimisation and bio-distribution of MSCs. The project has delivered important data and lays the groundwork for the future tailored production and use of MSCs with precise characteristics for particular applications.

We have also developed advanced technologies for 3D imaging and a novel GMP-compliant manufacturing process for producing MSC-based therapeutic product (ORBCEL-C™).

We are currently in the process of delivering an early phase clinical trial, exploring the safety and effect of our therapy on inflammation in patients with PSC and AIH. The MERLIN trial will continue after the formal end of the project.

We believe the project will ultimately impact on future research, manufacturing of MSCs for clinical use and cell therapies for patients with PSC and other inflammatory diseases.

1.4 *Impact, dissemination and exploitation.*

In MERLIN, WP10 was dedicated to maximising the value of the project by (i) communicating the project concept, progress and findings to a range of audiences and (ii) focusing on the future exploitation and commercialisation of MERLIN project results

1.4.1 Impact and exploitation

1.4.1.1 Overview

The MERLIN project has been looking at new ways to treat liver disease with mesenchymal stromal cells (MSCs) sourced from umbilical cord, specifically focusing on PSC and AIH as model diseases. As part of our work the team has looked at the effectiveness of MSC against inflammatory liver disease in laboratory models, explored mechanisms of action of MSC and studied optimum conditions for MSC production. We have also developed advanced technologies (3D imaging and processes for GMP-compliant manufacturing of MSCs).

Our work in the MERLIN project has culminated in the design and launch of a clinical trial, looking at safety and at the effect of MSC therapy on inflammation in patients with PSC and AIH. We hope that the MERLIN clinical trial will set the stage for future MSC-based therapies for PSC, AIH and other conditions involving inflammation.

1.4.1.2 Key Results

Some of the most significant findings from our results in the MERLIN project and our key achievements are set out below.

Efficacy:

- MSC reduce markers of liver damage and inflammation in pre-clinical models of inflammatory liver disease, as well as the number of inflammatory cells in damaged areas. MSC administration ameliorates PSC pathophysiology. Both bone marrow (BM) and umbilical cord (UC) derived MSCs show positive effects.
- MSC suppress inflammatory cells (lymphocytes) isolated from the peripheral blood and explanted livers of patients with PSC.
- Freshly thawed MSC are as effective as MSC used fresh from culture, or freshly thawed and washed (in murine models of liver damage).
- The route of infusion of UC MSC has no impact on therapeutic efficacy (MSC infused subcutaneously and intravenously were equally effective).

Mechanism of Action:

- MSCs injected under the skin do not migrate to other locations. Thus, the effects of MSC may be due to cytokines released by the cells (soluble mediators).
- The beneficial effect of MSCs on PSC is due, at least in part, to the extracellular vesicles (EVs) that MSCs secrete. Results suggest MSCs act as sensor of the surrounding inflamed environment, secreting EVs that block angiogenesis, limiting inflammation. The glycoprotein TIMP-1 plays a key role in the inhibition of angiogenesis by EVs.
- MSCs are phagocytosed (engulfed) by monocytic cells that subsequently migrate to other sites via the blood stream. Monocytic cells that have phagocytosed MSCs adapt an immunoregulatory phenotype and induce regulatory T cells.

Immune Response and Optimisation:

- UC MSCs are immuno-privileged, meaning they are less susceptible to immune recognition than MSCs from bone marrow.
- The immunogenic and immunomodulatory properties of MSCs are influenced by culture conditions. We have identified treatments that may enhance some properties of MSCs e.g. pre-treatment with a cytokine cocktail or with Budesonide. Notwithstanding this, in vivo experiments show good efficacy with untreated MSCs.

- Cell density and proliferation status of MSCs have a major effect on MSC phenotype, so MSC culturing protocols merit due consideration.

Biodistribution:

- MSCs injected under the skin do not migrate to other locations.
- There is evidence from imaging analysis that dispersal of sorted MSCs may be greater from injection site than wild-type MSCs.
- Notwithstanding the remote action of MSCs, with systemic delivery of MSCs we found a greater number of cells in the liver in CCl_4 injury and MDR2-KO injury mice, as compared to uninjured mice. This suggests injured livers may produce factors that attract/retain/promote survival of MSCs.
- MSCs are largely located in the lungs after administration, with an accumulation of dead MSCs in the liver 24h after infusion.

MSC Manufacture and Clinical Trial:

- Our work to date has allowed us to develop a process for MSC production from UC tissue that is consistent and aligned with regulatory requirements. NHSBT have manufactured specially selected MSCs (ORBCEL-C™, discovered by Orbsen Therapeutics) for delivery to patients in the trial.
- The MERLIN clinical trial for AIH and PSC patients opened for recruitment on 7 December 2018.
- The first patient was treated on 29 March 2019.

1.4.1.3 Exploitation and Exploitation Plans

\As will be noted from the above, MERLIN has resulted in a number of key innovations. Different partners will be exploiting the results of the project in different ways in the future. Exploitation can take many forms and may include: further research building on project results; commercial exploitation (new products or services, or the furtherance of existing ones), clinical exploitation and exploitation through the development of standards, guidelines or SOPs.

Some examples of the steps being taken/planned for the further exploitation of MERLIN results are set out below.

First, it is important to note, the partners will continue to run the MERLIN clinical trial after the project ends. This will ensure the continuation of the work started in MERLIN and forms an important part of the project's legacy.

Other ways in which MERLIN partners are exploiting and building on MERLIN include:

- UoB and ORB are involved in a new basket trial where multiple autoimmune conditions will be treated using MSCs. This will include Rheumatoid Arthritis and Crohn's disease (aim to open this trial in 2019).
- MW-ATTC collaboration (Midlands and Wales Advanced Therapy Treatment Centre), led by MERLIN Coordinator Professor Newsome has been awarded £7.3 million funding by Innovate UK, to deliver trials to help cell or gene therapies reach the clinical market.
- MW-ATTC collaboration: ORB were awarded £1.4M to establish GMP operations and develop ORBCEL-C for the POLARISE auto-immune basket trial.
- UoB (with industry partners) has been awarded £1.1 million by UK Research & Innovation (UKRI)'s Innovate UK, to investigate patients' experience of cell and gene therapies (PROmics study assessing the effect of novel cell therapies on patient symptoms and quality of life).
- ORB are collaborating with NHSBT to test ORBCEL-C in the Wellcome Trust REALIST clinical trial in patients with moderate to severe ARDS – with 3 patients treated in January 2019.
- EMC propose further research into long term immunomodulatory and regenerative effects, linking the MoA of MSC to morphology of MSC (modifying phenotype prior to administration to impact effect), and customising MSC therapy for particular indications, cells types and locations.
- EMC are involved in the Oxford-Aarhus-Erasmus MC consortium named MEPEP (Role of Mechynchymal Stem cells to regenerate renal graft function after ischemia reperfusion injury using normothermic ex-situ machine perfusion in pig transplantation; funded by the Lundbeck foundation, DK) where they use the gained knowledge of MERLIN to repair damaged kidneys.

- As inflammation is a well-known hallmark of cancer, UNIPD are planning to investigate the role of MSC derived EVs in mouse models of tumor progression (collaboration underway).
- UNIPD also plan to test the efficacy of MSC-EVs in the control of pathological angiogenesis characteristic for instance of eye diseases and tumours.
- SOPs and other controlled documents developed in the project will form the basis for similar developments and trials to be undertaken by NHSBT in collaboration with other academic and commercial partners. NHSBT has already started a follow-on collaboration with ORB testing ORBCEL-C in the Wellcome Trust REALIST clinical trial in patients with moderate to severe ARDS.
- BIO have further research collaborations underway and planned using their innovative 3D imaging technology (CryoViz™) e.g. metastatic cancer imaging in projects funded by the US National Cancer Institute through the Small Business Innovative Research schemes. Potential commercial opportunities include markets in regenerative medicine, cancer immune-therapy and gene expression.
- In terms of IP protection, ORB have filed a patent application relating to CD39+ cell selection and exosome production from ORBCEL (link here). UNIPD have also filed a patent application on new anti-angiogenic extracellular vesicles derived from MSCs (link here).
- Partners have also exploited their results by delivering numerous key conference presentations and achieving several peer reviewed publications (and a PhD).

1.4.1.4 Who will benefit?

MERLIN is a truly multi-disciplinary project, encompassing the generation of new knowledge about the bio-distribution and mechanism of action of MSCs, the development of advanced technologies (3D imaging and processes for GMP-compliant manufacturing), and the delivery of an early phase clinical trial. As such, the project will have an impact on future research, manufacturing of MSCs for clinical use and cell therapies for patients with PSC and other inflammatory diseases.

Patients with PSC and AIH, who currently have no treatment options, will benefit from the project. Through delivery of the Phase 2a trial, MERLIN will provide important safety and preliminary efficacy data for a novel MSC therapy. By focusing on understanding how the therapy works to fight inflammation, we are also learning important lessons that can be used in the future to develop MSC therapies for other types of liver disease and for inflammatory conditions in general. Patients will be informed of our results through our strong links to patient groups, in particular the PSC Support group in the UK. In addition to potential benefits for individual patients and their families, improving treatments for liver disease will also benefit healthcare systems through the reduction of costly liver transplant procedures and care costs associated with chronic liver disease. From a health economics perspective, MSC therapies for immune and inflammatory diseases offer potential for significant savings in the longer term.

Researchers in liver disease, immunology and regenerative medicine will benefit from the new knowledge generated by the project and the MERLIN trial. For researchers in liver disease and in the broader field of immunology and inflammatory conditions, MERLIN is providing new data on the use of MSCs to modulate immune response and to treat inflammation. The project will also have a significant impact in the field of MSC research. We are disseminating our findings widely through publications, presentations at scientific conferences and interactions with other projects. Importantly, MERLIN is part of a larger group of sister projects with many shared partners, providing an extensive network of researchers with whom we can share ideas and results. An additional benefit derived from the project will be the advancement of 3D imaging technology, which offers researchers new, better ways to carry out bio-distribution studies.

The European cell therapy industry will also benefit from the project. We have established GMP compliant processes and quality control tests to manufacture MSCs that meet the current (and emerging) regulations for MSC therapies. In addition, our research has explored the production of optimised MSCs. The project has delivered important data and lays the groundwork for the future tailored production and use of MSCs with precise characteristics for particular applications. In order to ensure that our innovations are of use to the industry, we have focused on ensuring that our advances meet regulatory requirements.

The MERLIN consortium is committed to building on the project in future collaborations, generating long-term research value and delivering societal and economic benefits through the development of advanced MSC therapies.

1.4.2 Dissemination

A summary of the dissemination activities carried out during the MERLIN project are set out below.

1.4.2.1 Website and online

The project website http://fp7merlin.eu/ was launched in month 1 of the project and has played a central role as a public dissemination tool and means of communication. 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://fp7merlin.eu/ - a general introduction to the project.
- Project Page http://fp7merlin.eu/project/ - a more detailed look at MERLIN, with a subpage describing the research plan and a subpage for publications (both project publications and related publications of interest).
- Partners Page http://fp7merlin.eu/partners/ - details of each partner in the consortium with links to partner websites.
- Media Page http://fp7merlin.eu/media-centre/ - basic project details, press releases, contact information for the Coordinator with a subpage for promotional materials http://fp7merlin.eu/media-centre/photos-videos-and-presentations/ where project flyers, brochures, newsletters and videos can be accessed.
- Related Projects Page http://fp7merlin.eu/related-projects/ - provides links to related and complimentary research projects.
- Contact Page http://fp7merlin.eu/contact-us/ – an online form which can be used to engage with the project team.
- News Page http://fp7merlin.eu/news/ – regular updates on the project and related developments.

The website has been regularly updated over the course of the Project.

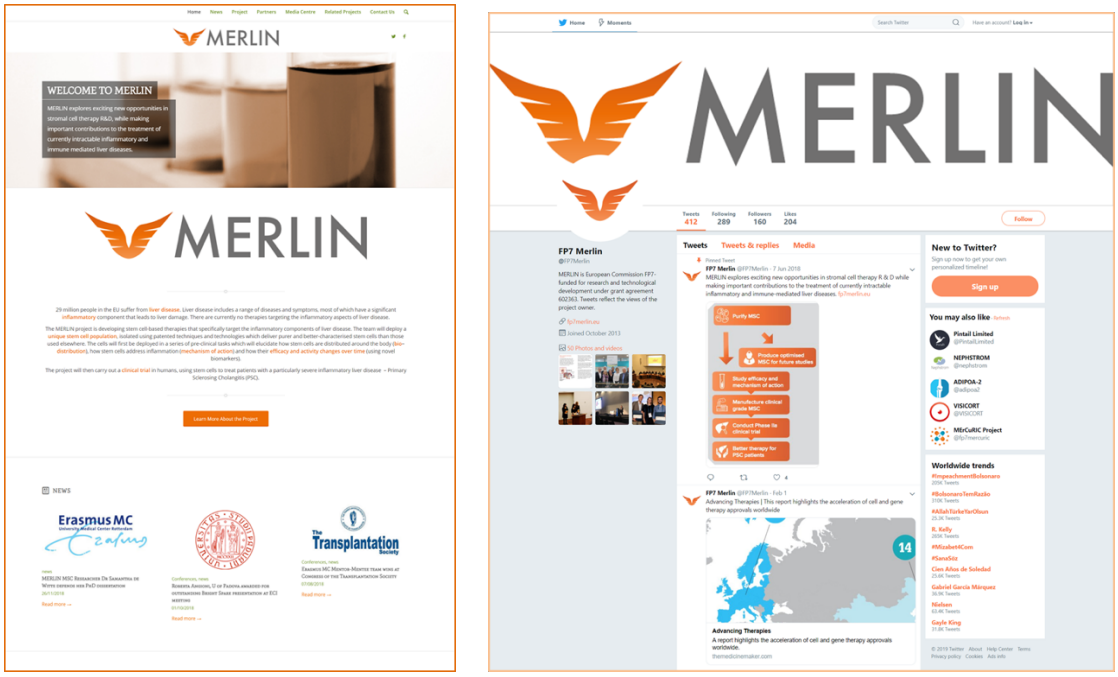


Figure 16 Screenshot of Project Homepage http://fp7merlin.eu/, Merlin Twitter Account

In addition to the website, MERLIN has an established social media presence, posting regularly on twitter (https://twitter.com/fp7merlin) and on Facebook (https://www.facebook.com/FP7MERLIN/).

1.4.2.2 Media

The project website has a dedicated media centre, which includes basic project facts, contact details for the Coordinator, the initial project press release and a factsheet from the British Liver Trust on PSC. There is also a promotional materials subpage where the project brochure, leaflet and newsletters can be accessed, together with 4 short videos featuring Merlin researchers explaining the project and the work being undertaken. The webpage makes key information about the MERLIN project easily accessible to interested journalists and to the general public

Press releases were issued at the start of the project. Press coverage included the Irish Times ("*Orbsen Therapeutics to take part in €6m liver disease clinical trial'*, Irish Times 22 April 2014), as well as coverage on Health Canal and My Science websites. Prof. Antonella Viola (UNIPD) was also interviewed by Italian broadcaster TG1.

A final press release has been issued, reporting that the first patient has been successfully treated in the clinical trial. The trial press release has so far been covered by the American venue, *Trial Site News*.

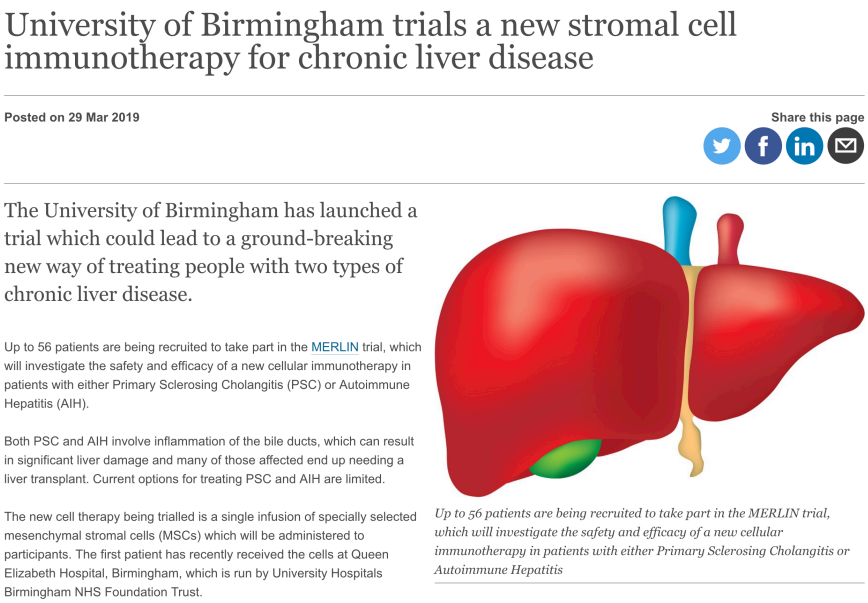


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Figure 17 UoB Press Release for start of clinical trial

1.4.2.3 Awards

Our young researchers have won awards and achieved their PhDs on the basis of their excellent work in MERLIN.

Erasmus MC
University Medical Center Rotterdam

MERLIN MSC Researcher Dr Samantha de Witte defends her PhD dissertation

26/11/2018 / in news / by merlin_admin

On Tuesday, November 20, 2018, Dr Samantha de Witte successfully defended her PhD. research at Erasmus Medical Center, Rotterdam, NL. The title of Dr de Witte's dissertation was "Probing the immunomodulatory potential of mesenchymal stromal cells".

MERLIN Coordinator Prof Phil Newsome, University of Birmingham, UK was one of Samantha's external examiners.

Congratulations, Samantha!

The Transplantation Society

Erasmus MC Mentor-Mentee team wins at Congress of the Transplantation Society

07/08/2018 / in Conferences, news / by merlin_admin

Erasmus Medical Center PhD student Jesus Sierra presented Merlin data at the Congress of the Transplantation Society in Madrid in July 2018. The title of Jesus' talk was: Monocytic cells phagocytose therapeutic mesenchymal stem cells, which induces polarization, relocation and immune regulation. Jesus Sierra in conjunction with Martin Hoogdujin, his mentor claimed the mentor-mentee award at the 27th International Congress of The Transplantation Society. This year's meeting was held in Madrid, Spain from June 30 to July 5, 2018.

Pictured below from left to right: Martin Hoogduijn, Erasmus Medical Center, Rotterdam, Marlies Reinders, President of the Dutch Transplantation Society, and Jesus Sierra, presenter and PhD student.

UNIVERSITAS STUDII PADUANI MCCXXII

Roberta Angioni, U of Padova awarded for outstanding Bright Spark presentation at ECI meeting

01/10/2018 / in Conferences, news / by merlin_admin

MERLIN PhD student Roberta Angioni of the Università degli Studi di Padova was selected for a "Bright Spark" presentation during the 5th European Congress of Immunology (ECI). The ECI meeting was held in Amsterdam on September 2-5, 2018.

Roberta remarks: "With my great pleasure, I was awarded with the "EFIS-Biolegend Bright Sparks Award for a presentation judged outstanding" for my presentation entitled "Extracellular Vesicles derived from licensed Mesenchymal Stem Cells: a tunable approach to regulate inflammatory angiogenesis (R. Angioni, S. Herkenne, C. Liboni, R. Sanchez-Rodriguez, B. Calì, G. Borile, A. Viola, Padova, Italy)".

1.4.2.4 Project Materials

During the course of the project we issued several project materials which were designed to engage with a broad audience.

At the start of the project we issued the project **brochure** and **flyer** setting out MERLIN's key aims. During the life of the project we issued 2 newsletters, proving updates on progress. The MERLIN website also hosts 4 project videos that feature Merlin researchers explaining the background to their research. A public version of the report for the MERLIN **Scientific Advisory Board** was prepared at the end of the first and second years of the project. These reports were made available on the project website.

Partners have been provided with hard copies of project materials for distribution locally and at events and conferences (including at events hosted by the International Society for Cellular Therapy (ISCT) and International Society for Stem Cell Research).

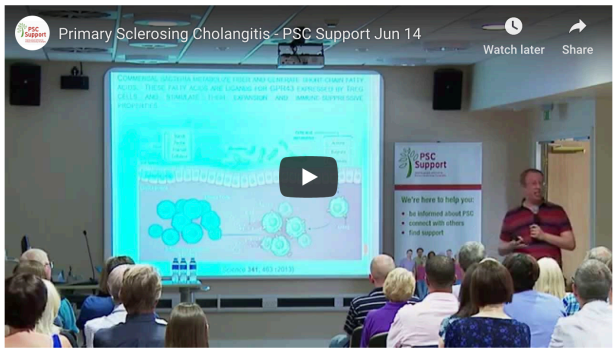
Finally, at the end of the project we issued the MERLIN **final flyer**, with a high level summary of the project's key achievements intended for a general audience. We distributed this, with cover letters, information about the project and press releases, to interested Members of the European Parliament.

All of the MERLIN project materials can be downloaded from the website: http://fp7merlin.eu/media-centre/photos-videos-and-presentations/.

Figure 18 Screenshot of some of the materials available on the project website.

1.4.2.5 Videos

The project produced a series of videos to explain our work and its value to the public.



1.4.2.6 Publications

We have connected with academics, researchers, industry and other projects through publications and presentations at key conferences and events.

Peer reviewed publications achieved in the project are set out below. Several additional publications are planned.

All publications are open-access.

Table 1 Summary of MERLIN peer review publications

D.O.I.	Title	Author(s)	Title of the periodical or the series	Number, date or frequency	Date of publication	Relevant pages
10.1038/leu.2016.33	Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1	L Zanotti et al	Leukemia	Vol. 30/Issue 5	01/05/2016	1143-1154
10.4414/smw.2015.14229	Mesenchymal stem cells: myths and reality	A Sarukhan , L Zanotti , A Viola	Swiss Medical Weekly	2015:145	23/12/2015	w14229
10.1186/s13287-017-0590-6	Cytokine treatment optimises the immunotherapeutic effects of umbilical cord-derived MSC for treatment of inflammatory liver disease	Samantha F. H. de Witte et al	Stem Cell Research & Therapy	Vol. 8/Issue 1	01/12/2017	140
10.1016/j.jcyt.2017.03.071	Aging of bone marrow– and umbilical cord–derived mesenchymal stromal cells during expansion	Samantha F.H. de Witte et al	Cytotherapy	Vol. 19/Issue 7	01/07/2017	798-807
10.1002/stem.2779	Immunomodulation By Therapeutic Mesenchymal Stromal Cells (MSC) Is Triggered Through Phagocytosis of MSC By Monocytic Cells	Samantha F.H. de Witte et al	Stem Cells	2018	01/02/2018	n/a
10.1016/j.jcyt.2018.05.005	Epigenetic changes in umbilical cord mesenchymal stromal cells upon stimulation and culture expansion	SAMANTHA F.H. DE WITTE et al	Cytotherapy	online	01/06/2018	online
10.1016/j.jprot.2017.07.012	Proteomic analysis of the secretome of human bone marrow-derived mesenchymal stem cells primed by pro-inflammatory cytokines	Elisa Maffioli et al	Journal of Proteomics	Vol. 166	01/08/2017	115-126

1.4.2.7 Conferences

MERLIN researchers have presented at numerous key conferences and events. A selection of these presentations is set out below.

- Dr Steve Elliman (ORB) introduced the project at the ISCT 2014 Conference in Paris on 22 April 2014, at a session entitled '*EU-funded projects in Cell Therapy*'.
- Dr Steve Elliman (ORB) gave a presentation '*Candace - The rapid development of an optimal stromal cell therapy*' at the 8th Annual UK MSC meeting in Galway, 29 - 30 Oct 2014.
- Dr M Garget and Dr D. Roy (BIO) made presentations on MERLIN overall project and on cryoimaging of whole mice for MSC bio-distribution as applied in the MERLIN project at the International Society for Cellular Therapy (ISCT) and International Society for Stem Cell Research (ISSCR) conferences in Las Vegas, USA (May 2015) and Stockholm, Sweden (June 2015), respectively.
- BIO team members presented at the ISCT Annual Conference on 25 September 2015 in Seville, Spain, title "*Cryo-Imaging System And Software For Assessing Msc Biodistribution In A Preclinical Model Of Liver Disease*". BIO also hosted a booth at the event, including MERLIN promotional materials.
- Dr Steve Elliman from Orbsen presented at the Keystone Symposia Stromal Cells in Immunity in Denver, Colorado (US) 7-12 February 2016. The title of his presentation was "*From CD362 to ORBCEL: the rapid development of a novel stromal cell therapy*".
- Researchers from Merlin project partner Erasmus MC made a number of presentations at the Dutch Transplantation Society Meeting held on 9 and 10 March 2016 in Groningen. These included a poster presentation about the Merlin project entitled "*Optimizing the immunogenicity and immunomodulatory properties of MSC* ", (S.F.H. de Witte et al).
- MERLIN was presented at the EASL Annual Meeting 2016 (Barcelona, 13-17 April 2016) in a bespoke session to highlight EU initiatives. Prof Phil Newsome (UoB, Coordinator of MERLIN) also presented at the special symposium on systemic inflammation in advanced chronic liver disease. Prof Newsome's presentation was entitled "*New concepts for targeting inflammation and immune dysfunction in advanced chronic liver disease".*
- Dr. Madhu Gargesha of BioInVision presented a paper titled "*MSC pre-treatments lead to better cell survival in mouse models of liver disease: A CryoViz Study*" at the American Association of Immunologists (AAI) 2016 Conference in Seattle, Washington, USA, May 13-16 2016.
- Researchers from EMC presented at the Nantes Actualités Transplantation (NAT) congress on Stem cells and immunology (9 June 2016). The presentation was entitled "*Optimizing immunomodulatory properties and immunogenicity of MSC for immunotherapy*" (Carla C. Baan and Martin J. Hoogduijn).
- Members of the Merlin team at EMC presented at the TTS 2016 Conference in Hong Kong on 20 August 2016 (presentation title: "*Optimizing the immunogenicity and immunomodulatory properties of MSC*") and at the MSC in Solid Organ Transplantation (MiSOT) meeting held in Regensburg on 27 October 2016.
- UoB researchers presented at the 7th European Club for Liver Cell Biology Meeting, (presentation: "*Identification of fibroblast activation protein expressing cells in injured liver*") held in the UK on 6 October 2016 and participated in the 10th UK Mesenchymal Stem Cell Meeting held in the UK on 5 December 2016.
- EMC researchers presented 2 papers at the Bootcongres, Dutch Transplantation Society, Groningen, Netherlands, on 8 March 2017. These were "*Ageing of bone marrow and umbilical cord derived MSC during culture expansion"* and "*In vivo tracking of live and dead mesenchymal stromal cells*".
- Dr Martin Hoogduijn from Erasmus MC presented at the meeting of The Transplantation Society (TTS) in Victoria, Canada on 24- 26th May 2017. The presentation was focused on MSC biology.
- Researchers from UNIPD presented a poster at the Congress of the Italian Society of Immunology, Clinical Immunology and Allergology, Bari Italy on 28 May 2017. The poster was entitled "*Extracellular Vesicles derived from licensed Mesenchymal Stem Cells: a tunable approach to regulate angiogenesis*".
- Samantha de Witte (Erasmus MC) presented "*Impact of culture expansion and inflammatory cytokine challenge on DNA methylation profiles in MSC"* at The European Society for Organ Transplantation (ESOT) Congress, Barcelona, Spain on 25 September 2017. Franka Luk (Erasmus MC) also presented "*Tracking and fate of umbilical cord-derived MSC after intravenous infusion in mice*".

- On 23 November 2017 Samantha de Witte (EMC) presented an abstract entitled "*Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells*" at the European Haematology Association (EHA), SWG Scientific Meeting on Shaping the Future of Mesenchymal Stromal Cells Therapy, Amsterdam, NL.
- Roberta Angioni (UNIPD) presented MERLIN's objectives and results during an invited talk entitled "*Mesenchymal stem cells and their extracellular vesicles in the control of pathological angiogenesis*" at the Pediatric Research Institute (IRP) meeting held on April 7, 2018, at the Villa Pace Park Hotel, Preganziol in Treviso, Italy.
- Head of Process Development at Orbsen Therapeutics, Dr Lisa O' Flynn attended the 3rd Annual Bioprocessing and Advanced Cellular Therapies Congress in Frankfurt, Germany. The meeting was held on the 29th-30th of May 2018. Dr Flynn gave an oral presentation entitled " *CD362 to ORBCEL: the rapid development of a novel stromal cell therapy* " in which the MERLIN clinical trial was highlighted.
- Erasmus Medical Center PhD student Jesus Sierra presented Merlin data at the 27th International Congress of The Transplantation Society in Madrid in July 2018. The title of the EMC presentation was "*Monocytic cells phagocytose therapeutic mesenchymal stem cells, which induces polarization, relocation and immune regulation"*. Jesus Sierra claimed the mentor-mentee award in conjunction with Dr Martin Hoogdujin, his mentor.
- MERLIN PhD student Roberta Angioni (UNIPD) presented "*Extracellular Vesicles derived from licensed Mesenchymal Stem Cells: a tunable approach to regulate inflammatory angiogenesis"* the 5th European Congress of Immunology (ECI). The ECI meeting was held in Amsterdam on September 2-5, 2018. Roberta Angioni received the EFIS-Biolegend Bright Spark award for her presentation.

1.4.2.8 Other

Other dissemination activities undertaken include engagement with patient organisations and groups. For example, the project has engaged with PSC patients through existing UoB links with PSC Support, a UK patient organization. MERLIN has been featured as a news item on the PSC Support site and on social media and UoB researchers have also attended PSC Support events.

Another important dissemination channel for the project has been EuroStemCell - the European-funded hub responsible for helping the European public understand stem cells. A briefing paper to introduce the project was prepared and delivered to the EuroStemCell team. MERLIN has been featured on the EuroStemCell website ((including an interview with MERLIN researcher Dr Steve Elliman from ORB about the commercial potential of stem cell therapies).

MERLIN researchers attended EU-MSC2 - a collaborative meeting for EU-funded stem cell research projects in Leiden on 7 and 8 September 2015 and on 12 and 13 September 2017. The EU-MSC2 meetings assembled twelve EU-funded mesenchymal stem cell-focused consortia, along with policymakers and other stakeholders in the sector.

1.4.3 Conclusion

In MERLIN we have reached out to our audience through the project website and social media, project flyers, newsletters and videos, issuing press releases, securing media coverage, presenting at conferences and events, publishing in peer review journals, liaising with patient organisations and connecting with related projects and researchers. We believe that our extensive dissemination programme, bringing MERLIN results and findings to a wide audience, will ensure the long-term legacy of the project.

1.5 *Website and contact details*

The MERLIN website is at www.fp7merlin.eu

Figure 19 Screenshot from MERLIN homepage

MERLIN can be contacted via the Project Coordinator:

Prof Philip Newsome,
Centre for Liver Research
5th Floor
Institute of Biomedical Research School of Immunity and Infection
College of Medical and Dental Sciences
University of Birmingham
Edgbaston, Birmingham B15 2TT
United Kingdom

Email: p.n.newsome@bham.ac.uk

MERLIN has brought together experts in stromal cell biology, inflammation and liver disease to work in collaboration with industry leaders in stromal cell manufacturing and advanced imaging, to develop a therapy for patients with PSC and AIH. The partners in the Project are set out below.

Figure 20 Members of the Merlin Team at a plenary meeting in Rotterdam

2. Use and Dissemination of Foreground

Section A

This section includes two templates:

- Template A1: List of all scientific publications relating to the foreground of the project.

- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

These tables are cumulative, which means that they should always show all publications and activities from the beginning until after the end of the project. Updates are possible at any time.

Please provide a list of all scientific publications relating to the foreground of the project, starting with the most important ones, in the table below.

2.1 *Template A1 – Publications*

D.O.I.	Title	Author(s)	Title of the periodical or the series	Number, date or frequency	Date of publication	Relevant pages	Open access is/will be provided to this publication
10.1038/leu.2016.33	Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1	L Zanotti , R Angioni , B Calì , C Soldani , C Ploia , F Moalli , M Gargesha , G D'Amico , S Elliman , G Tedeschi , E Maffioli , A Negri , S Zacchigna , A Sarukhan , J V Stein , A Viola	Leukemia	Vol. 30/Issue 5	01/05/2016	1143-1154	Yes
10.4414/smw.2015.14229	Mesenchymal stem cells: myths and reality	A Sarukhan , L Zanotti , A Viola	Swiss Medical Weekly	2015:145	23/12/2015	w14229	Yes

10.1186/s13287-017-0590-6	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 , Martin J. Hoogduijn	Stem Cell & Research Therapy	Vol. 8/Issue 1	01/12/2017	140	Yes
10.1016/j.jcyt.2017.03.071	Aging of bone marrow–and umbilical cord–derived mesenchymal stromal cells during expansion	Samantha F.H. 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 , Martin J. Hoogduijn	Cytotherapy	Vol. 19/Issue 7	01/07/2017	798-807	Yes
10.1002/stem.2779	Immunomodulation By Therapeutic Mesenchymal Stromal Cells (MSC) Is Triggered Through Phagocytosis of MSC By Monocytic Cells	Samantha F.H. de Witte , Franka Luk , Jesus M. Sierra Parraga , Madhu Gargesha , Ana Merino , Sander S. Korevaar , Anusha S. Shankar , Lisa O'Flynn , Steve J. Elliman , Debashish Roy , Michiel G.H. Betjes , Philip N. Newsome , Carla C. Baan , Martin J. Hoogduijn	Stem Cells	2018	01/02/2018	n/a	Yes
10.1016/j.jcyt.2018.05.005	Epigenetic changes in umbilical cord mesenchymal stromal cells upon stimulation and culture expansion	SAMANTHA F.H. DE WITTE , FLEUR S. PETERS , ANA MERINO , SANDER S. KOREVAAR , JOYCE B.J. VAN MEURS , LISA O'FLYNN , STEVE J. ELLIMAN , PHILIP N. NEWSOME , KARIN BOER , CARLA C. BAAN , MARTIN J. HOOGDUIJN	Cytotherapy	online	01/06/2018	online	Yes

10.1016/j.jprot.2017.07.012	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 Calì , Lucia Zanotti , Armando Negri , Antonella Viola , Gabriella Tedeschi	Journal of Proteomics	Vol. 166	01/08/2017	115-126		Yes

2.2 *Template A2 – Dissemination Activities*

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
1	Press releases	THE UNIVERSITY OF BIRMINGHAM	Pioneering Stem Cell Therapies to be Trialled in Birmingham	18/03/2014	University of Birmingham website	Scientific community (higher education, Research) - Industry - Civil society - Medias	UK
2	Web sites/Applications	THE UNIVERSITY OF BIRMINGHAM	Pioneering Stem Cell Therapies to be Trialled in Birmingham	18/03/2014	www.healthcanal.com	Scientific community (higher education, Research) - Industry - Medias	UK
3	Web sites/Applications	THE UNIVERSITY OF BIRMINGHAM	Pioneering Stem Cell Therapies to be Trialled in Birmingham	18/03/2014	www.myscience.org.uk	Scientific community (higher education, Research) - Industry - Medias	UK
4	Web sites/Applications	THE UNIVERSITY OF BIRMINGHAM	Pioneering PSC stem cell therapies to be trialled in Birmingham	19/03/2014	PSC Support website (UK PSC Patient Group)	Scientific community (higher education, Research) - Civil society - Medias	UK
5	Articles published in the popular press	ORBSEN THERAPEUTICS LIMITED	Orbsen Therapeutics to take part in ?6m liver disease clinical trial	22/04/2014	Irish Times	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias	Ireland
6	Web sites/Applications	ORBSEN THERAPEUTICS LIMITED	Orbsen Therapeutics In ?6M Research Funding Success	23/04/2014	NUIG News and Events (http://www.nuigalway.i	Scientific community (higher education, Research) - Industry -	Ireland

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
					e/about-us/news-and-events/)	Civil society - Policy makers - Medias	
7	Oral presentation to a scientific event	ORBSEN THERAPEUTICS LIMITED	EU funded projects in Cell Therapy	22/04/2014	ISCT 2014 Conference	Scientific community (higher education, Research) - Industry	International
8	Interviews	ORBSEN THERAPEUTICS LIMITED	Stem cells in the commercial world	15/05/2014	EuroStemCell (www.eurostemcell.org/story/stem-cells-commercial-world-interview-stephen-elliman)	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias	International
9	Oral presentation to a wider public	THE UNIVERSITY OF BIRMINGHAM	Current and Future Therapies for PSC	21/06/2014	PSC Support 2014 Birmingham Meeting	Civil society	UK
10	Oral presentation to a scientific event	ORBSEN THERAPEUTICS LIMITED	Cyndacel - The rapid development of an optimal stromal cell therapy	29/10/2014	8th Annual UK MSC Meeting	Scientific community (higher education, Research) - Industry	UK, Ireland
11	Web sites/Applications	THE UNIVERSITY OF BIRMINGHAM	EuroStemCell Connections - MERLIN description	15/12/2014	http://www.eurostemcell.org/connections/merlin	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias	International
12	Flyers	PINTAIL LTD	MERLIN - Year 1 Report	15/03/2015	www.fp7merlin.eu	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias	International

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
13	Flyers	PINTAIL LTD	Promotional Brochure and Flyer	12/05/2015	www.fp7merlin.eu	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias	International
14	Exhibitions	BIOINVISION INC	BioInVision Booth (with MERLIN promotional materials)	27/05/2015	ISCT 2015 Conference, Las Vegas	Scientific community (higher education, Research) - Industry	International
15	Exhibitions	BIOINVISION INC	BioInVision Booth (with MERLIN promotional materials)	24/06/2015	ISSCR 2015 Annual Meeting, Stockholm	Scientific community (higher education, Research) - Industry	International
16	Videos	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	EMC talk about their work on the MERLIN project	05/07/2015	https://youtu.be/y1bb-xWnsJo	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias	International
17	Oral presentation to a scientific event	THE UNIVERSITY OF BIRMINGHAM	MERLIN	07/09/2015	EU-MSC2 Meeting, Leiden	Scientific community (higher education, Research) - Industry	International
18	Posters	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	Dutch Transplantation Society Meeting poster presentation ?Optimizing the immunogenicity and immunomodulatory properties of MSC ?	09/03/2016	Groningen, Netherlands	Scientific community (higher education, Research)	Netherlands
19	Oral presentation to	BIOINVISION INC	Presentation "MSC pre-treatments lead to better cell survival in mouse models of liver disease: A	13/05/2016	Seattle, Washington, USA	Scientific community (higher education, Research)	International

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
	a scientific event		CryoViz Study" at the American Association of Immunologists (AAI) 2016				
20	Posters	UNIVERSITA DEGLI STUDI DI PADOVA	Poster at VIB conference Metabolism in Cancer and Stromal Cells title "Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1"	08/09/2015	Leuven, Belgium	Scientific community (higher education, Research)	International
21	Oral presentation to a scientific event	UNIVERSITA DEGLI STUDI DI PADOVA	Phd Students Meeting 2016, title of presentation "Mouse mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1"	13/07/2016	Milan, Italy	Scientific community (higher education, Research)	Italy
22	Exhibitions	UNIVERSITA DEGLI STUDI DI PADOVA	Exhibition/Poster: Mesechymal stem cells: novel therapeutical approaches	05/05/2016	Padova, Italy	Civil society	Italy
23	Oral presentation to a scientific event	UNIVERSITA DEGLI STUDI DI PADOVA	South Eastern European Immunology School, presentation title: "Mesenchymal Stem Cell Therapy: facts, hypotheses and challenges"	16/10/2015	Becici - Montenegro	Scientific community (higher education, Research)	Montenegro
24	Posters	ERASMUS UNIVERSITAIR MEDISCH	Poster at Molecular Medicine day Erasmus MC: Carla C. Baan and Martin J.	03/03/2016	Rotterdam, the Netherlands	Scientific community (higher education, Research)	Netherlands

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
		CENTRUM ROTTERDAM	Hoogduijn OPTIMIZING MSC FOR CELLULAR THERAPY				
25	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	Oral presentation Nantes Actualités Transplantation (NAT) congress on Stem cells and immunology: Carla C. Baan and Martin J. Hoogduijn "Optimizing immunomodulatory properties and immunogenicity of MSC for immunotherapy."	09/06/2016	Nantes, France	Scientific community (higher education, Research)	France
26	Flyers	THE UNIVERSITY OF BIRMINGHAM	Merlin Flash Report Year 2	02/06/2016	On-line	Scientific community (higher education, Research) - Industry - Civil society	International
27	Flyers	THE UNIVERSITY OF BIRMINGHAM	Merlin Newsletter Autumn 2016	26/10/2016	online http://fp7merlin.eu/merlin-newsletter-autumn-2016/	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias	International
28	Organisation of Conference	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	MSC in Solid Organ Transplantation (MiSOT) meeting	27/10/2016	Regensburg	Scientific community (higher education, Research)	German, international
29	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH	TTS 2016 Conference presentation, Optimizing the immunogenicity and	20/08/2016	Hong, Kong	Scientific community (higher education, Research)	International

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
		CENTRUM ROTTERDAM	immunomodulatory properties of MSC				
30	Interviews	UNIVERSITA DEGLI STUDI DI PADOVA	Eufactor Event, interview with Antonella Viola	30/09/2016	Padova, Italy	Civil society	Italy
31	Oral presentation to a scientific event	UNIVERSITA DEGLI STUDI DI PADOVA	EMBO meeting, presentation by Antonella Viola	27/10/2016	Heidelberg, Germany	Scientific community (higher education, Research)	International
32	Oral presentation to a scientific event	UNIVERSITA DEGLI STUDI DI PADOVA	Mechanisms of immune modulation by MSCs presentation	16/11/2016	Padova, Italy	Scientific community (higher education, Research)	International
33	Oral presentation to a scientific event	THE UNIVERSITY OF BIRMINGHAM	The 7th European Club for Liver Cell Biology Meeting, presentation: Identification of fibroblast activation protein expressing cells in injured liver	06/10/2016	Ascot, UK	Scientific community (higher education, Research)	International
34	Posters	THE UNIVERSITY OF BIRMINGHAM	Postdoctoral / Early Researcher Career Development And Training (PERCAT) Gala, UoB, poster: Identification of fibroblast activation protein expressing cells in injured liver	27/10/2016	Birmingham, UK	Scientific community (higher education, Research)	UK
35	Oral presentation to	THE UNIVERSITY OF	Participation in 10th UK Mesenchymal Stem Cell Meeting Stem Cell Therapy	05/12/2016	York, UK	Scientific community (higher education, Research)	UK

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
	a scientific event	BIRMINGHAM					
36	Exhibitions	THE UNIVERSITY OF BIRMINGHAM	Stem Cell Therapy at Meet the expert Thinktank, Birmingham Science Museum.	28/10/2016	Birmingham, UK	Civil society	UK
37	Oral presentation to a scientific event	THE UNIVERSITY OF BIRMINGHAM	EASL Annual Meeting 2016 bespoke session to highlight EU initiatives and Prof Newsome presentation at special symposium on systemic inflammation in advanced chronic liver disease "New concepts for targeting inflammation and immune dysfunction in advanced chronic liver disease?.	13/04/2016	Barcelona, Spain.	Scientific community (higher education, Research)	International
38	Oral presentation to a scientific event	BIOINVISION INC	ISCT Europe Meeting 2015 "CRYO-IMAGING SYSTEM AND SOFTWARE FOR ASSESSING MSC BIODISTRIBUTION IN A PRECLINICAL MODEL OF LIVER DISEASE"	23/09/2015	Seville, Spain	Scientific community (higher education, Research)	International
39	Exhibitions	BIOINVISION INC	BIO booth with Merlin promotional materials at the ISCT Europe Meeting 2015	23/09/2015	Seville, Spain	Scientific community (higher education, Research)	International

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
40	Oral presentation to a scientific event	ORBSEN THERAPEUTICS LIMITED	Dr Steve Elliman at Keystone Symposia Stromal Cells in Immunity. Title of Presentation ?From CD362 to ORBCEL: the rapid development of a novel stromal cell therapy?	07/02/2016	Denver, Colorado, USA	Scientific community (higher education, Research)	US, International
41	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	Invited lecture at the 10th UK Mesenchymal Stem Cell Meeting - Stem Cell Therapy	05/12/2016	York, UK	Scientific community (higher education, Research)	UK
42	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	Lecture at The Transplantation Society on MSC biology	24/05/2017	Victoria, Canada	Scientific community (higher education, Research)	Canada
43	Oral presentation to a scientific event	ORBSEN THERAPEUTICS LIMITED	Attendance at EU MSC2 Meeting	12/09/2017	Leiden	Scientific community (higher education, Research)	International
44	Posters	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	Erasmus MC, Science days of the Department of Internal Medicine, Poster: IMPACT OF CULTURE EXPANSION AND INFLAMMATORY CYTOKINE CHALLENGE ON DNA METHYLATION PROFILES IN MSC	31/01/2017	Antwerp	Scientific community (higher education, Research) - Civil society	Netherlands

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
45	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	Bootcongres, Dutch Transplantation Society, Presentation 1: Ageing of bone marrow and umbilical cord derived MSC during culture expansion	08/03/2017	Groningen, Netherlands	Scientific community (higher education, Research)	Netherlands
46	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	Bootcongres, Dutch Transplantation Society, Presentation 2: In vivo tracking of live and dead mesenchymal stromal cells	08/03/2017	Groningen, Netherlands	Scientific community (higher education, Research)	Netherlands
47	Posters	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	MOLMED day, Erasmus MC molecular medicine, Poster: IMPACT OF CULTURE EXPANSION AND INFLAMMATORY CYTOKINE CHALLENGE ON DNA METHYLATION PROFILES IN MSC	21/03/2017	Rotterdam, the Netherlands	Scientific community (higher education, Research)	Netherlands
48	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	European Society for Organ Transplantation (ESOT) Conference, Presentation 1: Impact of culture expansion and inflammatory cytokine challenge on DNA methylation profiles in MSC	24/09/2017	Barcelona, Spain.	Scientific community (higher education, Research)	International
49	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	European Society for Organ Transplantation (ESOT) Conference, Presentation 2: Tracking and fate of umbilical cord derived MSC	24/09/2017	Barcelona, Spain.	Scientific community (higher education, Research)	International

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
			after intravenous infusion in mice				
50	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	European Hematology Association (EHA), EHA-SWG Scientific Meeting on Shaping the Future of Mesenchymal Stromal Cells Therapy, Presentation 1: Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells	23/11/2017	Amsterdam	Scientific community (higher education, Research)	International
51	Flyers	PINTAIL LTD	Merlin Newsletter Winter 2017 - 2018 issued	16/02/2018	Online (and hard copies)	Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias	International
52	Posters	UNIVERSITA DEGLI STUDI DI PADOVA	Congress of the Italian Society of Immunology, Clinical Immunology and Allergology. poster "Extracellular Vesicles derived from licensed Mesenchymal Stem Cells: a tunable approach to regulate angiogenesis"	28/05/2017	Bari, Italy	Scientific community (higher education, Research)	Italian
53	Oral presentation to a scientific event	UNIVERSITA DEGLI STUDI DI PADOVA	"Mysenchymal Stem Cells and their Extracellular Vesicles in the Control of Pathological Angiogenesis" Roberta Angioni at meeting	07/04/2018	Treviso. Italy	Scientific community (higher education, Research)	Spain

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
			of the Pediatric Research Institute - IRP				
54	Oral presentation to a scientific event	ORBSEN THERAPEUTICS LIMITED	Dr Lisa O? Flynn at the 3rd Annual Bioprocessing and Advanced Cellular Therapies Congress ? CD362 to ORBCEL: the rapid development of a novel stromal cell therapy ? wherein the MERLIN clinical trial was highlighted	29/05/2018	Frankfurt, Germany	Scientific community (higher education, Research) - Industry	International
55	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	Jesus Sierra presented Merlin data at the Congress of the Transplantation Society: "Monocytic cells phagocytose therapeutic mesenchymal stem cells, which induces polarization, relocation and immune regulation". Jesus Sierra in conjunction with Martin Hoogdujin (mentor) claimed the mentor-mentee award.	01/07/2018	Madrid, Spain	Scientific community (higher education, Research)	International
56	Oral presentation to a scientific event	UNIVERSITA DEGLI STUDI DI PADOVA	Roberta Angioni ?Bright Spark? award at the 5th European Congress of Immunology (ECI), title "Extracellular Vesicles derived from licensed Mesenchymal Stem Cells: a tunable approach to regulate inflammatory angiogenesis	02/09/2018	Amsterdam	Scientific community (higher education, Research)	International

№	Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
57	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	Samantha de Witte, Erasmus MC, defence of Phd thesis	20/11/2018	Rotterdam	Scientific community (higher education, Research) - Civil society	Netherlands and International
58	Oral presentation to a scientific event	ERASMUS UNIVERSITAIR MEDISCH CENTRUM ROTTERDAM	Dr Martin Hoogduijn student lecture at EMC including MERLIN data	18/12/2018	Rotterdam	Scientific community (higher education, Research)	Netherlands

Section B -

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.

The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until the end of the project. ***Confidential/non-publishable entries must be clearly marked. Information under Section B that is not marked as confidential will be made available in the public domain by the Commission****.*

2.3 *Template B1 – Patents and IP Applications*

Type of IP Rights	Application reference(s) (e.g. EP123456)	Intellectual Property Organisation	Subject or title of application	Confidential	Foreseen embargo date	Applicant(s) (as on the application)	URL of application
Patents	USPCTIB2017000091	US	SDC-2 EXOSOME COMPOSITIONS AND METHODSOF ISOLATION AND USE	No		Orbsen Therapeutics Limited	https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2017122095&redirectedID=true
Patents	PCTIB2016057608	IB	NEW ANTI-ANGIOGENIC EXTRACELLURLAR VESICLES	No		Antonella ViolaMaurizio Muraca	https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2018109525&redirectedID=true

2.4 *Template B2 – Overview Table with Exploitable Foreground*

Confidential/non-publishable entries must be clearly marked. Information under Section B that is not marked as confidential will be made available in the public domain by the Commission

Type of exploitable foreground	Exploitable Foreground (description)	Confidential	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use or any other use	Patents or other IPR exploitation (licenses)	Owner & Other Beneficiary(s) involved
General advancement of knowledge	Our results clarify the MSC mechanism of action *in vivo.* First of all, we demonstrated that MSCs do not require homing to specific organ to control the inflammation. Additionally, we identify multiple soluble factors released by correctly licensed MSCs.	**NO**		Knowledge enabling further strategic research. The Foreground will be used to further our research agenda, to underpin new projects, and to contribute to the ongoing developments of new advanced therapies (e.g. the MW-ATTC centre, in which UoB and ORB are producing and distributing cells for advanced therapy companies and research teams).	Medical	ongoing	PCT/IB2016/057608	UNIPD, Antonella Viola, Maurizio Muraca
Commercial exploitation of R&D results	Software for analysing 3D biodistribution of cells in small animals	NO		MERLIN Biodistribution Explorer (MBE) software. This software is a key element of the BIO market offering, and has benefited directly from the work carried out in MERLIN. This	Life Sciences, healthcare, preclinical, medical	immediate	No	BioInVision

Type of exploitable foreground	Exploitable Foreground (description)	Confidential	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use or any other use	Patents or other IPR exploitation (licenses)	Owner & Other Beneficiary(s) involved
				enables BIO to offer enhanced products and services to the R&D communities, and to support further breakthroughs in life sciences.				
Commercial exploitation of R&D results	Hardware modifications for smart imaging based on tissue evidence, and high-throughput imaging	NO		Enhanced CryoVizTM system. CryoViz is the hardware associated with the MBE software listed above. CryoViz has benefited from the work in Merlin, including extensive use and configuration of the system to meet the various needs of the R&D teams. This positions BIO to offer an enhanced product in the market.	Life Sciences, healthcare, preclinical, medical	immediate	No	BioInVision
General advancement of knowledge	UoB will continue to run the MERLIN clinical trial after the project ends.	NO		Insights into efficacy and MoA of MSC are central to the development of new therapies, and new therapeutic components.	Life Sciences, healthcare, preclinical, medical	Already begun		UoB and ORB

Type of exploitable foreground	Exploitable Foreground (description)	Confidential	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use or any other use	Patents or other IPR exploitation (licenses)	Owner & Other Beneficiary(s) involved
	Further research and clinical studies building on MERLIN are also underway/planned, e.g.: New basket trial using MSCs. This will include Rheumatoid Arthritis and Crohn's disease MW-ATTC secured £7.3 million funding by Innovate UK, to deliver trials to help cell or gene therapies reach the clinical market. UoB (with partners) has been awarded £1.1 million by Innovate UK, to investigate patients' experience of cell and gene therapies ('PROMICS').			The results of the trial will open the door to a next-phase trial, and eventual access to the clinical market in PSC and similar types of disease. Exploitation is expected to involve both UoB and ORB. As noted on left, and below, these new insights are already underpinning important new R&D activities.				

Type of exploitable foreground	Exploitable Foreground (description)	Confidential	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use or any other use	Patents or other IPR exploitation (licenses)	Owner & Other Beneficiary(s) involved
Commercial exploitation of R&D results	Rapid development of processes, procedures for cord tissue processing, CD362+ cell selection/expansion from umbilical cord tissue.	NO		New IP filed around CD39+ cell selection and exosome production from ORBCEL. ORB are also collaborating with NHSBT to test ORBCEL-C in the Wellcome Trust REALIST clinical trial in patients with moderate to severe ARDS – with 3 patients treated in January 2019. Further research and clinical studies building on MERLIN are also underway/planned, e.g.: New basket trial with UoB (see above). MW-ATTC collaboration, ORB were awarded £1.4M for new cell production facility.	Advanced therapy production	Underway already	Key ORB IP (CD362-selection) was key enabling technology for MERLIN New CD39-selection and Exosome IP also WO-2017122095A1	ORB
General advancement of knowledge	New knowledge about mechanism of action, biodistribution, immunological	NO		Propose further research into long term immunomodulatory and regenerative effects, linking the MoA of MSC to	Cell R&D	Ongoing	N/A	EMC

Type of exploitable foreground	Exploitable Foreground (description)	Confidential	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use or any other use	Patents or other IPR exploitation (licenses)	Owner & Other Beneficiary(s) involved
	impact and optimisation of MSCs.			morphology of MSC (modifying phenotype prior to administration to impact effect), and customising MSC therapy for particular indications, cells types and locations.				
Commercial exploitation of R&D results + General advancement of knowledge	Manufacture of clinical grade MSC has given rise to new knowledge of regulatory requirements, SOPs and suite of regulatory documentation in NHSBT	NO		SOPs and other controlled documents will form the basis for similar developments and trials by NHSBT in collaboration with other academic and commercial partners. NHSBT has already started a second collaboration with Orbsen Therapeutics as a result of participating in the Merlin project (also involving producing MSCs from Cord Tissue for a clinical trial).	Cell therapy production	Ongoing	N/A	NHSBT
General advancement of knowledge	MERLIN foreground and new knowledge applied in a novel challenge	NO		EMC are involved in the Oxford-Aarhus-Erasmus MC consortium named MEPEP (Role of Mechynchymal Stem cells to regenerate renal graft function after ischemia	Medical	Ongoing	N/A	EMC

Type of exploitable foreground	Exploitable Foreground (description)	Confidential	Foreseen embargo date	Exploitable product(s) or measure(s)	Sector(s) of application	Timetable for commercial use or any other use	Patents or other IPR exploitation (licenses)	Owner & Other Beneficiary(s) involved
				reperfusion injury using normothermic ex-situ machine perfusion in pig transplantation; funded by the Lundbeck foundation, DK) where we use the gained knowledge of MERLIN to repair damaged kidneys.				

2.5 *Additional Template B2: Overview Table With Exploitable Foreground*

In the table, for each row, please provide a text to explain the exploitable foreground, in particular: - Its purpose - How the foreground might be exploited, when and by whom - IPR exploitable measures taken or intended - Further research necessary, if any - Potential/expected impact (quantify where possible).

1.	On the basis of the results collected in the context of the Merlin project and because our future research will focus on the extracellular vesicle (EV) therapeutic potential, we plan to start a collaboration with Prof. Maurizio Muraca, who is currently working at the Department of Women's and Children's Health SDB, at the University of Padova. Prof. Muraca is named inventor of the patent n° WO2013014691A1, which covers the immunomodulatory properties of EVs derived from MSCs. The findings that MSC affect angiogenesis paved the way for a further characterization of the molecular mechanisms regulating angiogenesis in other pathophysiological conditions.
2.	A software tool that enables the user to obtain and analyse 3D biodistribution (global and local) of cells within whole samples such as whole mice/rats. A modified and customized version of this tool could be sold as an add-on to future installs of CryoViz. Based on user feedback and additional findings, further research may be carried out and IP filed. The potential impact would be beyond stem cell regenerative medicine in areas such as imaging agent validation in metastatic cancer, cancer immunotherapy, nanoparticle theranostics, tumor burden assessment, cancer dormancy, etc.
3.	A modified version of CryoViz would enable faster scans due to automatic tissue detection, and high-throughput processing of organ samples. The foreground will be exploited by BIO in the next commercial iterations of CryoViz with additional features. Based on user feedback and additional findings, further research may be carried out and IP filed. The potential impact would be beyond stem cell regenerative medicine in areas such as imaging agent validation in metastatic cancer, cancer immunotherapy, nanoparticle theranostics, tumor burden assessment, cancer dormancy, etc

4.	The new basket trial (POLARISE) uses MSCs in three diseases, as outlined above. The potential foreground from this trial includes new therapies for a range of diseases. As elsewhere, this wil be protected by patents held by UoB or ORB. This trial is already being set up (funding secured); potential impact for challenging diseases (in terms of patient benefit and economic value) is very significant.
5.	MERLIN has been central to the success of UoB and ORB in securing 7M£ support from Innovate UK for the MWATTC initiative, which will greatly facilitate access to advanced therapies for UK patients. Many of the lessons learnt, technologies developed and systems created in MERLIN are used in MW-ATTC.
6.	NHSBT will use the processes and technologies developed and learnt in MERLIN in its ongoing and future production of cells for therapeutic purposes.

3. Report on Societal Implications

Replies to the following questions will assist the Commission to obtain statistics and indicators on societal and socio-economic issues addressed by projects. The questions are arranged in a number of key themes. As well as producing certain statistics, the replies will also help identify those projects that have shown a real engagement with wider societal issues, and thereby identify interesting approaches to these issues and best practices. The replies for individual projects will not be made public.

3.1 *Ethics*

1. Did your project undergo an Ethics Review (and/or Screening)?

No

If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final reports?

Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 2.2 'Work Progress and Achievements'.

2. Please indicate whether your project involved any of the following issues (tick box) :

RESEARCH ON HUMANS:

Did the project involve children?

No

Did the project involve patients?

Yes

Did the project involve persons not able to give consent?

No

Did the project involve adult healthy volunteers?

Yes

Did the project involve Human genetic material?

No

Did the project involve Human biological samples?

Yes

Did the project involve Human data collection?

Yes

RESEARCH ON HUMAN EMBRYO/FOETUS

Did the project involve Human Embryos?

No

Did the project involve Human Foetal Tissue / Cells?

No

Did the project involve Human Embryonic Stem Cells (hESCs)?

No

Did the project on human Embryonic Stem Cells involve cells in culture?

No

Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?

No

PRIVACY

Did the project involve processing of genetic information or personal data (e.g. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?

Yes

Did the project involve tracking the location or observation of people?

No

RESEARCH ON ANIMALS

Did the project involve research on animals?

Yes

Were those animals transgenic small laboratory animals?

Yes

Were those animals transgenic farm animals?

No

Were those animals cloned farm animals?

No

Were those animals non-human primates?

No

RESEARCH INVOLVING DEVELOPING COUNTRIES

Did the project involve the use of local resources (genetic, animal, plant etc)?

No

Was the project of benefit to local community (capacity building, access to healthcare, education etc)?

No

DUAL USE

Research having direct military use

No

Research having the potential for terrorist abuse

No

3.2 *Workforce Statistics*

Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

	Coordinator		WP leader		Sen Res./Phd		Student /RA		Other		Total		Of the total how many were new recruits for the project *	
Partner	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
UoB	1													
ORB			1			2				1	1	3		0
EMC			1	1		1		1		1			0	3
BIO			1		1				4	2			4	1
NHSBT			1			2				2			0	1
PT									2	3	2	3	0	0
UNIPD				1	1	5					1	6	0	0
TOTAL	1		4	2	2	10		1	6	9	3	12	4	5

3.3 *Gender Aspects*

5. Did you carry out specific Gender Equality Actions under the project?

No

6. Which of the following actions did you carry out and how effective were they?

Design and implement an equal opportunity policy.

No.

Set targets to achieve a gender balance in the workforce

No.

Organise conferences and workshops on gender

No.

Actions to improve work-life balance

No

Other:

7. Was there a gender dimension associated with the research content - i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

NO

3.4 *Synergies with Science Education*

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

Yes

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

Yes - the project involved dissemination of materials e.g. on the project website.

3.5 *Interdisciplinarity*

10. Which disciplines (see list below) are involved in your project?

Main discipline: *Medical Sciences.*

Associated discipline: *Clinical Medicine, Biological Sciences.*

3.6 *Engaging with Civil society and policy makers*

11a. Did your project engage with societal actors beyond the research community?

Yes

11b. If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

Yes- in communicating /disseminating / using the results of the project.

11c. In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?

No.

12. Did you engage with government / public bodies or policy makers (including international organisations).

Yes - in communicating /disseminating / using the results of the project.

13a. Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

Yes - as a primary objective (please indicate areas below multiple answers possible).

13b. If Yes, in which fields? *Public Health*

13c. If Yes, at which level? *International*.

3.7 *Use and dissemination*

14. How many Articles were published/accepted for publication in peer-reviewed journals?

Total: **7** (added automatically by portal). To how many of these is open access provided? ***7***

How many of these are published in open access journals?

How many of these are published in open repositories?

To how many of these is open access not provided?

Please check all applicable reasons for not providing open access:

publisher's licensing agreement would not permit publishing in a repository

no suitable open access journal available

no funds available to publish in an open access journal

15. How many new patent applications ('priority filings') have been made?

("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).

2

16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).

Trademark *0*

Registered design *0*

Other *0*

17. How many spin-off companies were created / are planned as a direct result of the project? *0*

Indicate the approximate number of additional jobs in these companies: *0*.

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

X Difficult to estimate / not possible to quantify.

None of the above / not relevant to the project.

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (FTE = one person working fulltime for a year) jobs:

**0*

X *Difficult to estimate / not possible to quantify*

3.8 *Media and Communication to the general public*

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

Yes

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

Yes

22. Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

X Press Release

Media briefing

TV coverage / report

Radio coverage / report

X Brochures /posters / flyers

X DVD /Film /Multimedia

Coverage in specialist press

Coverage in general (non-specialist) press

Coverage in national press

Coverage in international press

X Website for the general public / internet

X Event targeting general public (festival, conference, exhibition, science café)

23. In which languages are the information products for the general public produced?

Language of the coordinator

Other language(s)

X English