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CELL PRODUCTION

Development, functional characterization and validation of methodology for GMP-compliant manufacture of phagocytic macrophages: A novel cellular therapeutic for liver cirrhosis

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Abstract

Background aims. Autologous macrophage therapy represents a potentially significant therapeutic advance for the treatment of severe progressive liver cirrhosis. Administration of macrophages has been shown to reduce inflammation and drive fibrotic scar breakdown and tissue repair in relevant models. This therapeutic approach is being assessed for safety and feasibility in a first-in-human trial (MAcrophages Therapy for liver CirrHosis [MATCH] trial). *Methods.* We outline the development and validation phases of GMP production. This includes use of the CliniMACS Prodigy cell sorting system to isolate $CD14^+$ cells; optimizing macrophage culture conditions, assessing cellular identity, product purity, functional capability and determining the stability of the final cell product. *Results.* The GMP-compliant macrophage products have a high level of purity and viability, and have a consistent phenotypic profile, expressing high levels of mature macrophage markers 25F9 and CD206 and low levels of CCR2. The macrophages demonstrate effective phagocytic capacity, are constitutively oriented to an anti-inflammatory profile and remain responsive to cytokine and TLR stimulation. The process validation shows that the cell product in excipient is remarkably robust, consistently passing the viability and phenotypic release criteria up to 48 hours after harvest. *Conclusions.* This is the first report of validation of a large-scale, fully Good Manufacturing Practice–compliant, autologous macrophage cell therapy product for the potential treatment of cirrhosis. Phenotypic and functional assays confirm that these cells remain functionally viable for up to 48 h, allowing significant flexibility in administration to patients.

Key Words: *cell therapy, cirrhosis, GMP, macrophage, process validation*

Introduction

Liver cirrhosis is a major health problem in the Western world and a leading cause of mortality in the United Kingdom [1]. The disease is associated with a high level of morbidity due to the progressive tissue damage, fibrotic scarring and loss of liver function, and the only curative option for end-stage disease is liver transplantation. However, donor organ availability cannot meet demand, and often patients with end-stage liver disease are not eligible for transplantation. Those who do receive transplantation require lifelong immunosuppression with the increased health risks involved. Alternative therapies that prevent or delay the transition to terminal decompensated stages are urgently required [2].

The pathology of liver cirrhosis can be driven by numerous causative agents, including high alcohol consumption, obesity, metabolic disorders, viral infections or autoimmune disease, resulting in the progressive loss of healthy hepatocyte tissue and liver architecture, replaced by myofibroblast-derived fibrotic scarring

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[3,4]. It has been increasingly recognized that if the agents driving liver damage are removed (e.g., alcohol, viruses), then liver fibrosis can be at least partially reversible, enabling liver regeneration to occur [5]. Animal models of liver regeneration after experimental liver damage have shown that macrophages play a key role in the control and repair of fibrotic liver disease [6]. The macrophage compartment of the liver is complex and dynamic and can be significantly modulated by disease [7]. Resident Kuppfer cells and recruited hematopoietic-derived macrophages have distinct but overlapping functions; however, it is clear that liver repair can be therapeutically reproduced by administration of monocyte-derived macrophages but not undifferentiated bone marrow cells or monocytes [8]. Indeed, inflammatory monocytes can contribute significantly to pathology [9]. Macrophage therapy can resolve carbon tetrachloride (CCL_4)-mediated liver damage via decrease in myofibroblast levels and increased anti-inflammatory cytokine production [8]. Furthermore, macrophage-mediated matrix metalloprotease (MMP) release and phagocytosis is essential for fibrotic scar resolution [10]. Macrophages are also able to stimulate hepatic progenitor cells to proliferate and differentiate and replenish lost hepatocytes through Wnt and TWEAK signalling [11,12]. Adoptive macrophage therapy therefore offers a significant potential treatment strategy for patients with cirrhotic liver disease to promote resolution of fibrosis and stimulate resolution.

A number of studies have outlined GMP-compliant protocols for the generation of adoptive cell therapies using leukocytes including dendritic cells [13–15] natural killer cells [16] or cytotoxic T cells [17,18] *inter alia*. These studies largely harness the immune function of these cells. To date, few studies or trials have used macrophages for clinical cell therapy, focusing on either lung cancer [19] or bladder cancer [20,21]. One study of acute spinal cord injury involved use of autologous blood-derived macrophages [22], but this product was small scale (<2 million cells).

We have recently demonstrated that $CD14^+$ monocytes, collected by leukapheresis from cirrhotic donors, can be manufactured in a scalable manner into pro-resolution phenotype macrophages [23]. We are currently undertaking a first-in-human phase 0/1 safety and feasibility study to generate autologous $CD14^+$ cell–derived macrophages under Good Manufacturing Practice (GMP) for re-infusion into cirrhotic patients (MAcrophage Therapy for liver CirrHosis [MATCH] trial) [24].

The MATCH trial requires the development of a consistent, well-characterized, autologous macrophage cell product in doses of multiples of 10^8 cells. Here, we outline the development and validation for manufacturing of the MATCH product, including testing of GMP-grade media and growth factors for efficacy, analysis of cellular identity by multi-parameter flow cytometry, quantitative assessment of monocyte selection and macrophage purity and determination of a panel of markers which form the Release Criteria for the cell product. In addition, we examined the effects of monocyte cryopreservation on deriving macrophages for therapeutic use. Functional assays were conducted to quantify the phagocytic capacity of macrophages and their capacity for further polarization. This represents the first report of large-scale GMP-compliant macrophage manufacture and validation for first-in-human cell therapy of advanced liver cirrhosis.

Methods

Ethics and governance

Donor buffy coats as a source of healthy donor monocytes were provided by Scottish National Blood Transfusion Service (SNBTS) Blood Donor Centre, Edinburgh, United Kingdom, under SNBTS Sample Governance 13-12 and 14-02. For full-scale GMP process optimization and validation, peripheral blood mononuclear cells (PBMCs)were collected by leukapheresis in the SNBTS Clinical Apheresis Unit, Royal Infirmary of Edinburgh. Ethical approval was granted from the South East Scotland Research Ethics Committee 02. Informed consent for apheresis donation was obtained in accordance with the Helsinki Declaration.

Cell preparation

CD14 selection from PBMCs: PBMCs were separated from normal donor buffy coats by density centrifugation using Histopaque 1077 (Sigma). After washing, $CD14^+$ monocytes were isolated from the mononuclear cell fraction using CliniMACS GMP-grade CD14 microbeads and LS separation magnetic columns (Miltenyi Biotec). Briefly, cells were re-suspended to appropriate concentration in PEA buffer (phosphate-buffered saline [PBS] plus 2.5 mmol/L ethylenediaminetetraacetic acid [EDTA] and human serum albumin [0.5% final volume of Alburex 20%, Octopharma]), incubated with CliniMACS CD14 beads per manufacturer's instructions, then washed and passed through a magnetized LS column. After washing, the purified monocytes were eluted from the demagnetized column, washed and re-suspended in relevant medium for culture.

Isolation of $CD14^+$ cells from leukapheresis: PBMCs were collected by leukapheresis from cirrhotic donors who gave informed consent to participate in the study. Eligibility criteria were age range of 18 to 75 years, and cirrhosis was defined by any one of the following: previous liver biopsy confirming histologi-